VASCULAR EFFECTS OF BIOGENIC AMINES AND NEUROPEPTIDES

WITH REFERENCE TO CARCINOID PATIENTS

Kenneth Törnebrandt

Lund 1985

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41546224_0007

Organization
LUND UNIVERSITY
Department of Anesthesiology
University of Lund
S-221 85 LUND
Sweden

Document name
DOCTORAL DISSERTATION

Date of issue
1985 09 27

CODEN: LUMEDW/ (MEAN 1005)/1-172/(1985)

Author(s)
Kenneth Törnebrandt

Sponsoring organization

Title and subtitle

VASCULAR EFFECTS OF BIOGENIC AMINES AND NEUROPEPTIDES, with reference to carcinoid patients.

Abstract

Patients with metastatic carcinoid tumours were anaesthetized with a modified neurolept anaesthesia. This anaesthesia, without any specific blockade, together with careful monitoring and special avoidance of hypovolemia provided a safe procedure for these patients.

Carcinoid heart involvement (20%) in this type of patients were not as common or severe as earlier reported. Pulmonary hypertension was rare (13%) and slight. There was no correlation between perioperative changes in circulation, respiration, or degree of heart involvement and changes in plasma 5-hydroxytryptamine (5-HT) levels.

Pharmacological characterization of isolated human mesenteric vessels revealed that α_1-receptors were predominantly responsible for the achieved contraction in the arteries, whereas α_2-receptors were predominant in the veins. 5-HT also caused a contraction of the mesenteric vessels with a higher sensitivity than norepinephrine (NE) in the arteries but a similar intrinsic activity in the veins. Among the neuropeptides tested, neuropeptide Y (NPY) contracted both arteries and veins with an intrinsic activity about 10 times higher than that found for NE and 5-HT. Calcitonin gene-related peptide, bradykinin, and substance P (SP) regularly dilated both arteries and veins.

The 5-HT_2 receptor antagonist, Ketanserin, caused a partly irreversible inhibition of the 5-HT induced contraction. Spantide, a SP-receptor antagonist, interacted with the SP-induced dilatation in a not purely competitive way.

Both NE and 5-HT potentiated each other's contractile effects in the arteries, but not in the veins. NPY potentiated the effect of NE primarily through a presynaptic effect.

The demonstrated interaction between NE, 5-HT, and NPY may be a part of an integrated modulating system regulating the blood circulation, and this might explain why blockade of the effect of only one substance is not beneficial for patients with carcinoid tumours producing several different hormones.

Key words
Carcinoid disease and anaesthesia; carcinoid heart; human mesenteric arteries and veins; adrenoceptors; vascular serotonin receptors; neuropeptides and vascular function; norepinephrine and serotonin potentiation; neuropeptide Y and norepinephrine interaction.

Classification system and/or index terms (if any)

Supplementary bibliographical information

Language
English

ISSN and key title

ISBN

Recipient's notes

Number of pages
172

Price

Security classification

DOKUMENTDATABLAD enl SIS 61 41 21

Distribution by (name and address)
Kenneth Törnebrandt, Department of Anesthesiology, University of Lund, S-221 85 LUND, Sweden.

I, the undersigned, being the copyright owner of the abstract of the above-mentioned dissertation, hereby grant to all reference sources permission to publish and disseminate the abstract of the above-mentioned dissertation.

Signature K. Törnebrandt Date 1985 08 28

VASCULAR EFFECTS OF BIOGENIC AMINES AND NEUROPEPTIDES

with reference to carcinoid patients

Kenneth Törnebrandt
leg läk, Mlm

Akademisk avhandling

som för avläggande av medicinsk doktorsexamen
vid Medicinska Fakulteten vid Universitetet i Lund
kommer att offentligen försvaras
å Barnklinikens föreläsningssal, Lasarettet Lund,
fredagen den 27 september 1985 kl 11.15.

ERRORS

pg	ln		should be
I:1	last	substance	substance P
I:4, legend	3	(a)	(d)
	11	<p<	>p>
	13	<p<	>P>
	16	<p<	>P>
I:5	8	191.2	19.1
III:6 and III:10			changing of figures
IV:9	4	16.4±3.0	18.1±3.6
IV:16	33	and veins	⊢——⊣
IV:19	15	403-408	1077-1080
V:12	19	(27, 28)	(28, 29)

From the Departments of Anaesthesia, Histology, and Surgery,
University of Lund, S-221 85 Lund, Sweden

VASCULAR EFFECTS OF BIOGENIC AMINES AND NEUROPEPTIDES
with reference to carcionid patients

Kenneth Törnebrandt

Lund 1985

Printed in Sweden
Studentlitteratur
Lund 1985

To ANNA

Give us the tool
and we will finish the job

Sir Winston C Churchill

The present thesis is based on the following papers:

I. Törnebrandt K, Nobin A, Ericsson M, Thomson D: Circulation, respiration and serotonin levels in carcinoid patients during neurolept anaesthesia. Anaesthesia 38: 957-967 (1983).

II. Törnebrandt K, Eskilsson J, Nobin A: Heart involvement in metastatic carcinoid disease. Clinical Cardiology 8: 000-000 (1985).

III. Törnebrandt K, Nobin A, Owman Ch: Pharmacological characterization of alpha-adrenergic receptor subtypes mediating contraction in human mesenteric arteries and veines. Blood Vessels 22: 000-000 (1985).

IV. Törnebrandt K, Nobin A, Owman Ch: Serotonergic smooth muscle receptors in human mesenteric arteries and veins. Submitted.

V. Törnebrandt K, Nobin A, Owman Ch: Vasomotor effects of nine neuropeptides on isolated human mesenteric blood vessels. Submitted.

VI. Hanko J, Törnebrandt K, Hardebo JE, Kåhrström J, Nobin A, Owman Ch: Neuropeptide Y induces and modulates vasoconstriction in intracranial and peripheral vessels of animals and man. Submitted.

In the text the papers are referred to by their Roman numerals.

ABBREVIATIONS AND CALCULATED HEMODYNAMIC VARIABLES

CGRP	Calcitonin gene-related peptide
5-HT	5-Hydroxytryptamine (serotonin)
(n)	Number of experiments
NE	Norepinephrine
NPY	Neuropeptide Y
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PPP	Platelet-poor plasma
SD	Standard deviation
SEM	Standard error of mean
SP	Substance P
VIP	Vasoactive intestinal polypeptide

MAP	Mean arterial blood pressure (normal, 70-105 mmHg)
CO	Cardiac output (normal, 4-8 litres/minute)
CI	Cardiac index (normal, 2.5-4 litres/minute/sq m body surface area)
SV	Stroke volume (normal, 60-100 ml)
MPAP	Mean pulmonary artery pressure (normal, 15-20 mmHg)
PCW	Pulmonary capillary wedge pressure (normal, 6-12 mmHg)
CVP	Central venous pressure (normal, 0-5 mmHg)
SVR	Systemic vascular resistance (= (MAP - CVP) x (1/CO); normal, 10-15 mmHg/litre minute/sq m)
LVSW	Left ventricular stroke work (= SV x (MAP - PCW) x 0.0136; normal, 45-75 g m)
PVR	Pulmonary vascular resistance (= (MPAP - PCW) x 1/CO); normal, < 3.12 mmHg/litre minute/sq m)
RVSW	Right ventricular stroke work (= SV x (MPAP - CVP) x 0.0136; normal, 10-15 g m)

CONTENTS

BACKGROUND.....9

AIMS OF THE PRESENT STUDY.....15

MATERIAL.....16

METHODS.....18

RESULTS.....24

DISCUSSION.....37

CONCLUDING REMARKS.....45

ACKNOWLEDGMENTS.....46

REFERENCES.....47

Paper I

Paper II

Paper III

Paper IV

Paper V

Paper VI

BACKGROUND

Endocrine abdominal tumours may arise from different organs such as the pancreas, the adrenals, and the intestinal tract. Patients with these tumours demand special anaesthetic care (Katz et al., 1981). Most tumours originating from the pancreas are insulinomas, which require a close regulation of blood sugar levels during the peri-operative period. Patients with pheocromocytomas from the adrenal medulla are difficult to manage due to intermittent excessive catecholamine release and abrupt decline in this release after removal of the tumour (Katz et al., 1981). Carcinoid tumours are usually composed of endochrine cells usually of the enterochromaffin type, and found in the gastrointestinal tract, biliary tract, ovary, pancreas, and the lung (Jager and Polk, 1977). In the small intestine and appendix they are the most common tumours. The tumours from the appendix rarely metastazise, but those from other locations often metastasize to the regional lymph nodes and the liver (Moertel et al., 1961; Mårtensson et al., 1983; Mårtensson et al., 1985). The carcinoids form and release a series of biological active substances, such as 5-hydroxytryptamine (5-HT), bradykinin, prostaglandins, and substance P (SP) (Oates et al., 1964; Håkanson et al., 1977; Jager and Polk, 1977; Jaffe, 1979; Nobin et al., 1982; Emson et al., 1984). 5-HT has a pressor activity in situations with a low sympathetic tone (Pagé and McCubbin, 1953) as in patients during anaesthesia. This hypertensive effect is augmented by both positive inotropic and chronotropic actions on the heart (Buccino et al., 1967). The bradykinins are potent vasodilators and may be responsible for the so called bradykinin shock (Dery, 1971), to which also SP can contribute (cf. Pernow, 1983; Zinner et al., 1984). In addition to the circulatory effects, bronchospasm is likely to occur due to the effects of 5-HT and different peptides (Dery, 1971; Jager and Polk, 1977).

Patients with carcinoid tumours are hazardous to manage during anaesthesia (Stone and Donnelley, 1960; Jones, 1962; Malavoud et al., 1970; Dery, 1971; Mason and Steane, 1976; Kleine et al., 1977; Desmonts et al., 1978; Miller et al., 1978; Koehler et al., 1978; Patel and Dalal, 1980; Jones and Knight, 1982). Severe complications with tachycardia, hyper- or hypotension and bronchospasm have been reported to occur in more than 50 % of patients during anaesthesia (Mason and Steane, 1976). The systemic effects of liberated hormones should occur only when liver metastazing has happened, since the hormones otherwise are eliminated by the passage through the

liver. In spite of this, severe hypertension during anaesthesia has been reported also in patients lacking liver metastases (Jones and Knight, 1982). This hypertension can be explained by the release of an amount of hormones from the primary tumour that exceed the capacity of the liver.

In longstanding carcinoid disease right heart valvular stenosis and insufficiency have been reported to occur in up to 50 % of the patients. (Callahan et al., 1982; Howard et al., 1982; Forman et al., 1984). The most common cardiac lesions are pulmonic stenosis and tricuspid regurgitation which probably are equally frequent (Roberts and Sjoerdsma, 1964; Trell et al., 1973), although a predominance of pulmonic stenosis has been found earlier (Thorson et al., 1954). The cardiac lesions have been claimed to be one of the most important lethal factors in carcionid patients (Thorson et al., 1958; Roberts and Sjoerdsma, 1964; Trell et al., 1973; Costello, 1975) although contradictory findings also are available in the litterature (Howard et al., 1982). 5-HT has been proposed as the major factor (Thorson et al., 1954; Roberts and Sjoerdsma, 1964; Trell et al., 1973) responsible for the fibrous deposits between the internal elastic membrane and the endothelium in the heart. However, animal trials with chronic 5-HT infusions have failed to produce the lesions (Waldenström, 1968). The pathophysiological role of other substances released from the tumour (e.g. bradykinin and SP) is still unknown (Jager and Polk, 1977). The left heart is protected by the 98 % hormonal elimination through the lungpassage, and left sided lesions are rare (Trell et al., 1973, Strickman et al., 1982).

The main effects on the cardiovascular system of the active substances released from the carcinoids are exerted on smooth muscle cells in the blood vessel wall. The smooth muscle tone is due to the influence and balance between the sympathetic and parasympathetic nervous system, including the recently discovered peptidergic systems, and circulating vasoactive substances.

The classical transmitter in the sympathetic nervous system is norepinephrine (NE) (von Euler, 1951) which exerts its action on vascular adrenoceptors (α and β receptors). It is however, today recognised that a large portion of the sympathetic nerves also contain neuropeptide Y (NPY) (Lundberg et al., 1982; Lundberg et al., 1983; Uddman et al., 1985). The release of NE from postganglionic sympathetic nerves is regulated not only by the central nervous system, but also by many receptor-mediated mecha-

nisms located on or near the postganglionic nerve terminales (see Starke, 1977; Langer 1981). The sympathomimetic vasoconstriction are today recognized to be mediated by α-adrenoceptors (Ahlquist, 1948; Furhgott, 1972). These α-adrenoceptors are pharmacologically classified into α_1- and α_2- subtypes regardless of their anatomical localization (Starke, 1981). Postjunctionally, a mixed population of both α_1- and α_2-adrenoceptors exists (Starke, 1981; Langer and Shepperson, 1981), whereas the prejunctional receptors are of the α_2-subtype (Langer, 1981; Starke, 1981) mediating an 'inhibitory feedback loop' (Majewski and Starke, 1984).

The catecholamines also affect the β-adrenoceptors, eliciting strong vasodilatation (cf. Owman, 1980). This vasodilatation is mediated by β_2-receptors in the peripheral vascular bed and β_1-receptors in intracranial and coronary vessels. On the other hand, stimulation of prejunctional β-adrenoceptors may modulate the release of NE (Langer, 1981; Majewski, 1983).

The cholinergic, parasympathetic, innervation of the peripheral circulation has a less widespread distribution than the adrenergic fibres. The occurrence of neurogenic, cholinergic vasodilatation is debated in human skeletal muscles, only anecdotical for the gastro-intestinal tract, and not yet identified in human skin (Rowell, 1981). The cholinergic vasodilation can instead be elicited by glandular stimulation which leads to humoral liberation (adenosin mono- (AMP), di- (ADP), and tri-phosphate (ATP), histamine, bradykinin). This vasodilation may cooperate with strong vasodilatory effects of vasoactive intestinal polypeptide (VIP), which has a widespread perivascular distribution, partly coexisting with acetylcholine (Lundberg et al., 1980). Another possibility is that liberated acetylcholine acts on muscarinic prejunctional adrenergic nerve terminals and inhibits NE-release (Vanhoutte and Shepherd, 1983). The postjunctional cholinergic dilation requires an intact endothelium (Furchgott, 1981).

Apart from being isolated from carcinoid tumours (enterochromaffin cells) 5-HT is primarily present in platelets, and in the central nervous system (Dahlström and Fuxe, 1965; Nobin and Björklund, 1973; Steinbusch, 1984). Recently 5-HT has been shown to be stored in mesenteric and pial vascular nerves (Costa et al., 1982; Griffith and Burnstock, 1983). The physiological role of 5-HT on the cardiovascular system is not clear. 5-HT can both

stimulate and inhibit various smooth muscles and autonomic nerves (Pagé and McCubbin, 1953) and the response differs between species, animals of the same species and even in successive tests in the same individual (Essman, 1977-78; Douglas, 1980). The 5-HT induced contraction may be due to direct activation of 5-HT receptors of the vascular endothelial or smooth muscle cells, amplification of the response to other vasoconstrictor agents, activation of α-adrenoceptors in the vascular wall, or displacement of endogenous NE from nerve endings (Fozard, 1976) with subsequent activation of α-adrenoceptors pre- or postjunctionally.

The 5-HT vascular receptors are probably functionally divided into several subgroups (van Nueten et al., 1981; Humphrey, 1983). The 5-HT_2 receptor subtype probably mediates the contraction in most blood vessels (Edvinsson et al., 1978; Shepherd and Vanhoutte 1979; van Neuten et al., 1981; Arneklo-Nobin and Owman, 1985). This assumption is mainly based on the inhibition of contraction by the 5-HT_2 antagonist, ketanserin (van Nueten et al., 1981; Cohen et al., 1983; Arneklo-Nobin and Owman, 1985).

5-HT can also augment the vasoconstrictor responses to other agonists such as NE, angiotensin II, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and histamine (de la Lande et al., 1966; Scroop and Walsh, 1968; van Nueten et al., 1981; Moreland and Bohr, 1983; Seabrook and Nolan, 1983). The amplifying effect of 5-HT can be blocked by ketanserin in concentrations that do not depress the contractile effect of the agonists used (van Nueten and Vanhoutte, 1984).

The vasodilator effect of 5-HT has been observed mainly in the intact organism, but can also be demonstrated in vitro on fairly large blood vessels (Edvinsson et al., 1978; Vanhoutte, 1978; Vanhoutte et al., 1981; Cohen et al., 1983; Mecca and Webb, 1984). The 5-HT induced vasodilatation is presumably due, at least in part, to 5-HT_1 receptor activation on endothelial cells and adrenergic neurons, by release of inhibitory transmitters from peptidergic nerves, and/or inhibitory effects on the vascular smooth muscle cells (Vanhoutte, 1984).

Immunocytochemistry has revealed a variety of neuropeptides, such as SP, VIP, NPY, and calcitonin gene-related peptide (CGRP), localized in perivascular nerve fibres. Such nerves seem to be present in all mammalian species including man, though the density is different in various vascular

beds. SP was first described by von Euler and Gaddum (1931). It is a very potent vasodilating compound in humans shown by intraarterial administration in the forearm (Eklund et al., 1977). SP is a more potent histamine liberator than most other vasoactive peptides (Johnson and Erdös, 1973; Erjavec et al., 1981), so at least some of the dilatory effect of SP can be mediated by a release of histamine from the mast cells. It is likely that SP mediates the local vasodilatation induced during antidromic stimulation of sensory nerves (Lembeck and Holzer, 1979; Hökfelt et al., 1982). On the other hand, it acts in high doses as a vasoconstrictor of the mesenteric vein in rabbits and the portal vein in rats (Hellstrand and Järhult, 1980), and injection of SP into the lateral brain ventricle of the rat causes increased mean arterial blood pressure (Unger et al., 1980). Recently, it has been possible to synthesize different SP-analogues with specific SP receptor blocking characteristics (Leander et al., 1981; Pernow, 1983). Of these, (D-Arg^{1}, D-Pro^{2}, D-Trp^{7,9}, Leu^{11})SP has been shown to be a potent SP-antagonist without agonistic activity (Rosell et al., 1983a) and (D-Arg^{1}, D-Trp^{7,9}, Leu^{11})SP (Spantide) to have an even higher affinity to the SP-receptor in the guinea-pig ileum and the rat urinary bladder (Rosell et al., 1983b).

VIP is also a potent vasodilator (Larsson et al., 1976; Hellstrand and Järhult, 1980; Uddman et al., 1981). It appears to be present together with acetylcholine and peptide histidine isoleucine (PHI) in perivascular nerves in various mammals including man (Lundberg et al., 1980; 1984; Ekblad et al., 1984a). PHI and VIP relax the guinea-pig trachea in vitro (Lundberg et al., 1984). VIP is a strong transmitter candidate for the atropine-resistant vasodilatation in various tissues (Lundberg et al., 1980; 1984).

NPY was initially isolated from porcine brain (Tatemoto et al., 1982). It has been found that NPY-immunoreactive nerve fibres are particularly numerous in the walls of blood vessels (Furness et al., 1983; Stjernquist et al., 1983; Sundler et al., 1983; Terenghi et al., 1983; Uddman et al., 1984), and that NPY co-exists with NE in sympathetic nerve fibres (Lundberg and Tatemoto, 1982; Lundberg et al., 1982; Uddman et al., 1985). NPY has been described to cause vasoconstriction in intracranial and peripheral vessels from various animals (Lundberg et al., 1982; Edvinsson et al., 1983; Owman et al., 1984; Hardebo et al., 1985), and recent evidence indicates that NPY co-operates with NE in vascular reactions both at a prejunctional and postjunctional level (Edvinsson et al., 1984a,b; Ekblad et al., 1984b).

Application of recombinant DNA and molecular biological techniques have revealed the existence of previously unknown neuropeptides, such as CGRP. This peptide has been localized in sensory neurons as well as in perivascular nerves of the brain and peripheral organs of laboratory animals and man (Rosenfeld et al., 1983). CGRP has been shown to have strong vasodilatory actions (Brain et al., 1985), which are exerted both through direct influence on the vascular smooth musculature and by inhibition of neurogenic vasoconstriction (Fisher et al., 1983; Brain et al., 1985; Hanko et al., 1985).

The majority of these biological active amines and peptides are also well recognized to occur in, and to be released from the cells of endocrine abdominal tumours (Alumets et al., 1983; Mårtensson et al., 1983; 1985). Thus, patients with these tumours may present rather complex problems during anaesthesia due to release of these substances. It is desirable to increase our knowledge about the frequency and type of both circulatory and respiratory disturbances. This should help us to choose the best anaesthetic technique, to early recognise complications, and to handle them adequately. One approach could be a combination of studies in vivo and in vitro, where the isolated effect of the amines and peptides on blood vessels can be analysed.

AIMS OF THE PRESENT STUDY

The present study was undertaken to analyse:

- the circulatory and respiratory functions during anaesthesia in patients with malignant carcinoid tumours

- the blood and plasma concentrations of one of the active substances (5-hydroxytryptamine) released from the carcinoid tumour cells during different phases of anaesthesia and surgery

- the changes in hemodynamics and blood hormone concentrations during flush episodes

- the value during anaesthesia of different precautions undertaken pre- and peroperatively

- the frequency and characteristics of heart involvement in longstanding carcinoid disease

- the vascular effects in vitro of biogenic amines and neuropeptides known to occur in, and to be released from endocrine abdominal tumours

- the pharmacological characteristics of α- and β-adrenoceptors as well as serotonergic and peptidergic receptors in the human mesenteric vascular system

- the interaction on the vascular smooth muscle cells in vitro of norepinephrine and serotonin, and norepinephrine and neuropeptide Y, respectively

- the antagonistic properties in human mesenteric blood vessels of recently developed antagonists to 5-HT and substance P receptors.

MATERIAL

Paper I:

Four female and 10 male consecutive patients (aged 36 - 71 years, mean 58) with carcinoid tumours of ileal or caecal origin treated at the Department of Surgery, Lund, were studied during 16 operations. All patients had liver metastases. They were normotensive and, except in one patient who had a cardiac dysrhythmia, there was no history of cardiac disease or bronchiolar obstruction. The aim of the operations were to reduce the tumour mass. Temporary dearterialization (10), ileocaecal resection and debulking of liver metastases (1), debulking of liver metastases (1), right hemicolectomy and small bowel resection (1), ileotransversostomy (1) and exploratory laparotomy (2) were performed.

Paper II:

Nine women and 7 men (aged 43 - 72 years, mean 62) with carcinoid tumours of ileal or caecal origin were selected from the Department of Surgery where they were subjected to surgery or liver embolisation. All patients had liver metastases, and the carcinoid syndrome with facial flushing and diarrhoea was present in 12 patients. At the time of investigation, the patients had suffered from symptoms of their carcinoid desease for 11 - 181 months (mean, 61 months). Autopsy was performed by a pathologist on 2 of the 8 patients who died during the follow up period. The patients had elevated levels of 5-HT in PPP and whole blood as well as elevated excretion of 5-hydroxyindoleacetic acid in urine. SP in plasma was measured in 10 patients and raised levels were registered in 7 of them.

Papers III, IV, and V:

Small segments of mesenteric arteries and veins were obtained from a total of approximately 260 men and women, aged 20 -70 years, undergoing surgery due to non-endocrine ventricular or intestinal malignancy. The patients were given different combinations of the following anaesthetics: morphine chloride and scopolamine bromide, droperidol, fentanyl, pentothal sodium, succinylcholine, pancuronicum bromide, dinitrous oxide and oxygen.

Paper VI:

Small segments of mesenteric arteries and veins were received from 20 patients undergoing intestinal resection of non-endocrine tumours. Human pial arteries (diameter, 0.3-0.6 mm) were received during temporal lobe resection due to brain tumour operations in 3 patients. The patients were anaesthetized as mentioned in papers III - V. Middle cerebral arteries, mesenteric arteries and veins were removed from 6 cats. The basilar, mesenteric, central ear arteries and mesenteric veins were taken from 20 rabbits. Both the cats and rabbits were exsanguinated under pentobarbitone anaesthesia.

METHODS

Anaesthetic technique (paper I)

All patients were premedicated with droperidol (5 mg) and fentanyl (0.1 mg) intramusculary 45 minutes before arriving in the operating room. The first five cases also received levopromazine (12.5 mg) intramusculary in the evening before surgery and at the time of premedication, in accordance with the routine in our hospital (Carlsson, 1969). In the induction room the patient was given 1 litre of Ringer's lactate solution to ensure adequate filling pressures during induction of anaesthesia. In the meantime an arterial and a triple lumen thermistor pulmonary artery catheter (Swan Ganz^{R}) were inserted under local anaesthesia. Anaesthesia was induced by droperidol 0.15 mg/kg and fentanyl 0.015 mg/kg given intravenously, and by inhalation of 50 % nitrous oxide in oxygen over a period of 5 minutes. The ventilation was assisted at the end of that period. After muscular relaxation with pancuronium (1 mg/kg) and spraying with lidocaine (80 mg), the patients were nasotracheally intubated and connected to a volume controlled ventilator (Siemens-Elema, Servo 900B) with an end tidal CO_2 analyser 930 (Olsson et al., 1980). The patients were ventilated with 65 % nitrous oxide in oxygen with a minute volume to give an end tidal tension of CO_2 of about 4 kPa. Supplemental doses of fentanyl (0.5 mg/hour in divided doses) and pancuronium were given as needed. Blood losses were replaced unit for unit, and maintenance fluid, 5.5 % glucose with 80 mmols sodium/litre, were given to keep the cardiac pressures to the upper normal range and a urinary output of 1 ml/kg/hour (see Table I).

Table I. Anaesthetic drugs used in the study.

Levopromazine (Nozinan^{R}, Leo Rhodia)
Droperidol (Dridol^{R}, Janssen Leo Pharma)
Fentanyl (Leptanal^{R}, Janssen Leo Pharma)
Pancuronium bromide (Pavulon^{R}, Organon)
Lidocaine (Xylocain^{R}, Astra)
Pentobarbitone (Mebumal^{R}, Abbott)

Registration and measurements during anaesthesia (paper I)

The electrocardiogram, peripheral arterial pressure and pulmonary pressure were continously registered from the time of induction. After intubation airway pressures and flow, as well as end tidal tension of CO_2 and CO_2 production, were monitored using the Servoventilator and, together with the circulatory variables, registered on an eight-channel ink-writer (Mingograph 81, Siemens-Elema). CO was measured and haemodynamic variables were calculated; especially before induction, before surgery, during a period of the operation when no or little surgical stimulation was applied ('during surgery'), and 'during flushing'.

Biochemical methods (papers I and II)

Before the operation a catheter was placed in the right hepatic vein via the femoral vein, and blood samples were collected at different times (I). In paper II blood samples were taken in a peripheral vein. 5-HT (I and II) was determined in whole blood and PPP with high pressure liquid chromatography (HPLC) with electrochemical detection (Nobin et al., 1982). 5-hydroxyindoleacetic acid in urine (I and II) was measured by a spectrophotometric method (Macfarlane et al., 1956). SP in plasma was determined by radioimmunoassay (Emson et al., 1984).

Heart catheterization (paper II)

Fourteen of 16 patients were invasively monitored via an arterial and a triple lumen pulmonary catheter inserted under local anaesthesia. During catheter withdrawal pressure recordings were obtained from the pulmonary artery, the ventricle and atrium.

Echocardiography (paper II)

An M-mode study was made in 4 patients (Organon Teknika Echocardiovisor 01). In 11 patients a two-dimensional echocardiography study was obtained with a mechanical sector scanner (ATL, USA; 3 MHz transducer). Contrast echocardiography (Lieppe et al., 1978; Howard et al., 1982) was performed in 5 patients. Doppler-cardiography with a pulsed Doppler unit was also used (ATL, USA; Miyatake et al., 1982). Clinical cardiac examination, chest x-ray and electrocardiogram were performed on all patients.

Vascular preparations for in vitro studies (papers III, IV, V, and VI)

All vessels were removed with an atraumatic technique with an ischaemic period of less than 20 sec for the human vessels. After removal they were immediately transferred to a cold, modified Krebs-Ringer solution (see Table II) and kept at +4 ^{o}C in a refrigerator. The experiments were performed on the same day or the subsequent 2 days.

Table II. Buffer solutions used in the study.

(III, IV, V) In mM concentration: NaCl 118; KCl 4.5; $CaCl_2$ x 2 H_2O 2.5; $MgSO_4$ x 7 H_2O 1.0; $NaHCO_3$ 25; KH_2PO_4 1.0; glucose 6.0.

(VI) In mM concentration: NaCl 118; KCl 4.7; $CaCl_2$ x 2 H_2O 1.0; $MgSO_4$ x 7 H_2O 1.2; $NaHCO_3$ 24.8; KH_2PO_4 1.2; glucose 5.6.

Vessel diameter (papers III, IV, V, and VI)

Small pieces of the vessels were taken to fixation in Bouin's solution and stained in hematoxyline and eosin in transverse sections of 0.005 mm. Light microscopic examination was used for measuring wall thickness from which the passive load the vessels should be given in the organ bath could be estimated (Frank, 1920).

Recording of mechanical activity (papers III, IV, V, and VI)

Five-mm long circular vascular segments were carefully prepared free from surrounding connective tissue, and mounted between two L-formed metal holders (diameter, 0.05 or 0.1 mm) in a 50-ml (III), 5-ml (IV, V) or 2.5-ml (VI) organ bath (Fig. 1) containing modified Krebs-Ringer solution (see Table II). Bath temperature was thermostatically maintained at 37 ^{o}C and the buffer solution was continuously aerated with a mixture of 95 or 88.5 % O_2, and 5 or 11.5 % CO_2 (depending on bath volume) giving a pH of 7.40 (range 7.35-7.45). The vascular tension was recorded isometrically with Grass FT03C force-displacement transducers and registered on a Grass model 79D polygraph. The vessels were given an initial passive tension of 5-30 mN, depending on vessel caliber and type of vessel (see III), and allowed to equilibrate for 60-90 min before the experiments were started.

Agonists were added to the bath in a cumulative way (van Rossum and van den Brink, 1963) since this gave identical (III) or similar (IV) results as fractionated application. When the amines were tested, the bath contained 10^{-6} M each of cocaine and normetanephrine to block neuronal and extra neuronal uptake respectively (Iversen, 1967). When contractile response was tested, 10^{-6} M propranolol was present to block any β-receptor mediated dilatory effect (cf. Edvinsson et al., 1978). For testing the dilatory effect, the vessels were given an active tone by $PGF_{2\alpha}$, uridine triphosphate or potassium and the contractile α-receptor effect was blocked with phenoxybenzamine (10^{-6} M) when the amines were tested.

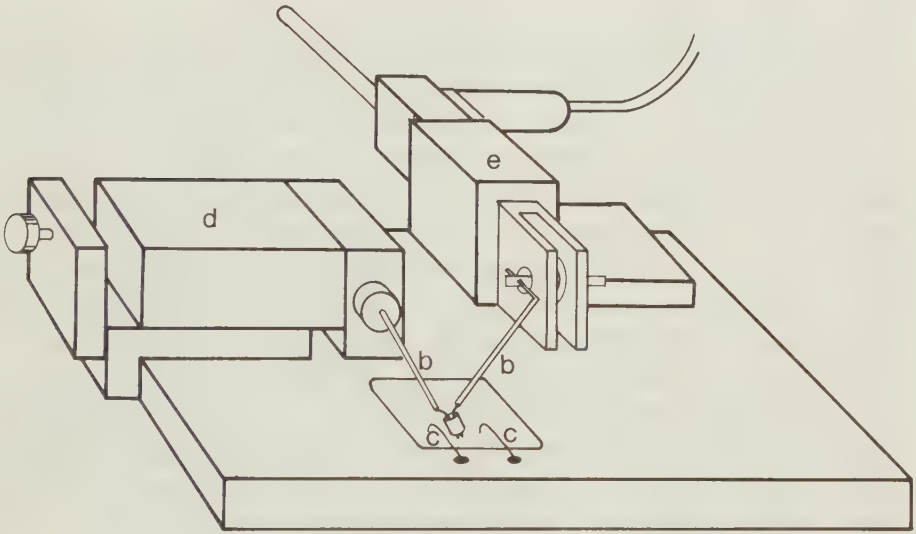


Fig. 1. Schematic drawing of the myograph: (a) organ bath, 2.5 or 5.0 ml; (b) metal holders, diameter 0.05 or 0.1 mm; (c) platinum electrodes, diameter 0.3 mm; (d) displacement unit with micrometer screw; (e) force-displacement transducer which was connected to a Grass model 79 D polygraph. The buffer solution in the bath was heated by circulating, thermostatically heated water inside the myograph block. A gas mixture was continously aerating the buffer solution at the bottom of the bath (Adapted from Högestätt et al., 1983).

Electrical field stimulation (paper VI)

The vessels were stimulated via platinum electrodes (0.3 mm in diameter and 4 mm apart) from both sides (Fig. 1). The stimulation was given as a 10-sec train of square wave pulses every 6 min using a Grass S44 stimulator with an additional external power supply of 45 V. Voltage was continuously measured on a oscilloscope.

Calculations

For hemodynamic values (I) see 'Abbreviations and calculated hemodynamic variables'. Formulae are from cardiopulmonary Analysis Programs for Hewlett Packard 67 and 97 Calculators, modified after Civetta (see Neglén, 1980). The lung mechanical values, inspiratory resistance and compliance of the respiratory system were calculated according to formulae given by Johnsson et al. (1975).

All concentration-response curves were plotted either in absolute values or in terms of relative vasomotor response in a semi-logarithmic form. These curves were used to estimate the maximum response (E_{Am}) and the concentration of agonists giving half maximum response (EC_{50}), which in paper VI was expressed as the negative logarithm of the EC_{50} value (pD_2). When antagonists were used, their competitive nature was established in Schild plots (Arunlakshana and Schild, 1959). In these the logarithm for the dose ratio minus 1, plotted against the negative logarithm for the concentration of the antagonist, should fit a straight line with a slope of unity. The intercept of this line with the abscissa represents the pA_2 value, which is the negative logarithm for the antagonist concentration which doubles that concentration of the agonist to produce a given effect (Schild, 1947). The corresponding mean values for the receptor-antagonist complex (K_B) was calculated (Furchgott, 1972).

Student's t-test for unpaired and paired observations was used to compare mean values. Spearman's rank coefficient was used for testing correlation (I). Regression lines were obtained by the method of least squares and the values are given with 95 % confidence intervals. Other values are given as mean $\pm$ SEM. Statistical significance was accepted for $p < 0.05$.

Pharmacological substances (papers I, II, III, IV, V, and VI)

See table I - V.

Table III. The α-receptor agonists and antagonists used in the study.

	Agonists	Antagonists
Mainly α_1-type	Methoxamine hydrochloride (Burroughs, Wellcome)	Prazosin (Pfizer)
	L-Phenylephrine hydrochloride (Sigma)	
$\alpha_1 \sim \alpha_2$	L-Arterenol hydrochloride (Sigma)	Phenoxybenzamine hydrochloride (Sigma)
	Epinephrine hydrochloride (Sigma)	Phentolamine (Ciba-Geigy)
Mainly α_2-type	Oxymetazoline chloride (Draco)	Rauwolscine hydrochloride (Roth)
	Clonidine hydrochloride (Draco)	Yohimbine hydrochloride (Sigma)
	L-Isoproterenol hydrochloride (Sigma)	Piperoxan hydrochloride (Rhône-Poulenc)

Table IV. Peptides used in the study.

Calcitonin gene-related peptide (Peninsula)
Leu-enkephalin (CRB)
Lys-bradykinin (CRB)
Met-enkephalin (CRB)
Neuropeptide Y (Peninsula)
Neurotensin (Peninsula)
Somatostatin 14 (CRB)
Substance P (Sigma)
Vasoactive intestinal polypeptide (CRB)

Table V. Various drugs used in the study

Atropine sulphate (Alcon)
Carbamylcholine chloride (Aldrich)
Cocain hydrochloride (ACO)
5-hydroxytryptamine creatinine sulphate (Sigma)
Guanethidine (Ciba-Geiby)
Ketanserin tartrate (Janssen)
Normetanephrine hydrochloride (Sigma)
Propranolol hydrochloride (Inderal^R, ICI)
Prostaglandin $F_{2\alpha}$ (Amoglandin^R, Recip)
R 55 667 (Ritanserin^R, Janssen)
(D-Arg^1,D-Trp^{7,9}, Leu^{11})-SP (Spantide^R Bachem)
Terbulatine sulphate (Bricanyl^R, Draco)
Tetrodotoxin (Sanyo)
Uridine triphosphate (Sigma)

RESULTS

Hemodynamics during anaesthesia (paper I)

Pretreatment with a non-specific 5-HT blocker, levopromazine, in carcinoid patients did not change the hemodynamic variables (HR, MAP, CI, MPAP, PCW, CVP, SVR, LVSW, PVR, and RVSW) before induction of anaesthesia, and the reaction to induction was practically the same. 'During surgery' the patients pretreated with levopromazine had a higher degree of peripheral vasodilatation (lower SVR, $p < 0.05$) and a higher HR ($p < 0.05$), together giving a higher CI ($P < 0.01$) than for the other patients (Table VI). PVR was the same in both groups, but MPAP was lower ($p < 0.05$) in the group of levopromazine pretreated patients. 'During flush' these patients increased more in MPAP ($p < 0.05$) and CI ($p < 0.05$) which gave a higher stroke work for both the left (LVSW; $p < 0.05$) and right (RVSW; $p < 0.05$) side of the heart.

Table VI. Mean (+SEM) values of cardiovascular variables in non-pretreated patients and levopromazine pretreated patients during surgery and during flush.

	DURING SURGERY		DURING FLUSH	
	Non-treated patients (n = 11)	Levopromazine pretreated patients (n = 5)	Non-treated patients (n = 4)	Levopromazine pretreated patients (n = 4)
HR, beats/min	79 (10.5)	94 (8.5)^{o}	102 (11)*	100 (4.7)
MAP, mm Hg	94.7 (2.3)	88.0 (8.0)	94.0 (15.0)	96.5 (11.1)
MPAP, mm Hg	14.1 (1.1)	9.8 (1.0)^{o}	12.8 (1.8)	16.8 (1.9)*
CI, litres/min	2.8 (0.2)	3.6 (0.2)^{oo}	3.2 (0.2)	4.7 (0.3)^{oo}*
SVR, mm Hg/litre min/sq.m	21.4 (1.9)	13.0 (0.9)^{o}	18.8 (3.5)	11.4 (2.5)
PVR, mm Hg/litre min/sq.m	1.4 (0.3)	1.0 (0.06)	1.4 (0.15)	1.1 (0.24)
LVSW, g m	76.6 (8.5)	70.8 (14.3)	71.5 (14.7)	108 (9.5)^{o}*
RVSW, g m	8.4 (3.2)	6.6 (1.9)	7.7 (2.4)	16.3 (2.4)^{o}*

Differences from values between groups during surgery and flush: ^{o} $0.05 > p > 0.01$; ^{oo} $0.01 > p > 0.001$

Differences from values within groups during surgery: * $0.05 > p > 0.01$

Postoperative course (paper I)

Four patients were extubated directly after surgery. Slow awakening is known for carcinoid patients due to raised cerebral 5-HT concentrations (Dery, 1971) and 11 patients were mechanically ventilated for the first 12 hours postoperatively. No sedative drugs were necessary during this period. The patients were thereafter extubated without complications. Only one patient had a complicated postoperative period. Due to postoperative hypovolemia the patient went into a carcinoid shock with hypotension, oliguria, oedema and adult respiratory distress syndrome 12 hours after the operation. The patient could leave the intensive care unit after 3 days of symptomatic treatment without sequele.

Respiration (papers I and II)

None of the 30 patients (I + II) had any history of bronchial constriction. One patient (II) suffered from shortness of breath due to diffuse interstitial fibrosis. During anaesthesia (I) the O_2 and CO_2 tension in blood remained normal in all patients. In one patient signs of bronchiolar constriction were seen. There were rapid fluctuations in both inspiratory and expiratory resistance measured at the start of expiration. The values were calculated to change from 1.8 to 2.7 kPa/litre/sec (normal, 1.6, SD 4) and from 2.0 to 9.0 kPa/litre/sec (normal, 2.1 SD 7), respectively. However, the changes were of no clinical importance since no alterations in blood gasses were seen and no treatment necessary.

Heart involvement in carcinoid disease (paper II)

Carcinoid heart lesions were found in 3 of 16 patients (19 %), but only one of these had subjective symptoms associated with the heart disease. In 2 of these 3 patients right ventricular overload together with either a tricuspid valve leaflet insufficiency or a mild pulmonary stenosis were registered. In the third patient thickened, fibrous tricuspid valve leaflets were found at autopsy, which indicates an insufficiency of the leaflets.

Four patients (25 %) had a slight pulmonary hypertension. No left-sided heart lesions were observed.

In paper I no patient had a pressure gradient over the pulmonary valves indicating a stenosis, or pulmonary hypertension.

Clinical course (paper II)

Eight patients died during the follow-up period. The mean time from the heart investigation to death was 15 months (range 1-39 months). Nothing from the autopsy protocol (n = 2) or clinical data indicated that the probable course of death was cardiac failure, but the carcinoid tumour growth per se.

Biochemistry and flushing (papers I and II)

5-hydroxytryptamine. Of 30 patients only 3 had approximately normal concentrations of 5-HT in PPP (I + II). Among the 13 patients with elevated blood 5-HT concentrations 10 patients had a history of flushing (I). During operations 6 of these patients flushed. 5-HT was measured at 13 flushing episodes in different patients and increased levels of more than 50 % were registered 6 times. There was no statistic correlation between the 5-HT concentrations or variations, and the levels or variations in either HR, MAP, or MPAP. No correlation between the measured high levels of 5-HT in PPP and the degree of heart involvement was found.

Substance P. Of 3 patients with cardiac carcinoid involvement, the SP value was raised in one patient, normal in one and not measured in the third patient.

Pharmacological in vitro studies (papers III, IV, V, and VI)

General characteristics of the vessels. The diameter of the human mesenteric arteries was 0.4-0.9 mm, and of the veins 0.5-1.7 mm. An initial load of 20-35 mN, tested with NE, gave maximum contraction in these vessels (III). Storage at +4 ^{o}C in a buffer solution did not change the response to NE with a storage time tested up to 48 h (III) or to 5-HT tested up to 24 h (IV). Control studies with NE (III) and 5-HT (IV) showed no significant change in EC_{50} and E_{Am} values over the test period, although an inconsistent degree of tachyphylaxis occurred for 5-HT.

α-receptor mediated contraction in mesenteric arteries (paper III)

The EC_{50} values for the sympathomimetic contractions were in the order oxymetazoline > epinephrine > norepinephrine > phenylephrine > methoxamine > isoproterenol. The differences were all significant ($p < 0.001$) (Fig. 2a). Both phentolamine and prazosin displaced the NE concentration-response curves towards higher concentrations without reduction of E_{Am}. Schild plots gave straight lines with slopes not significantly different from

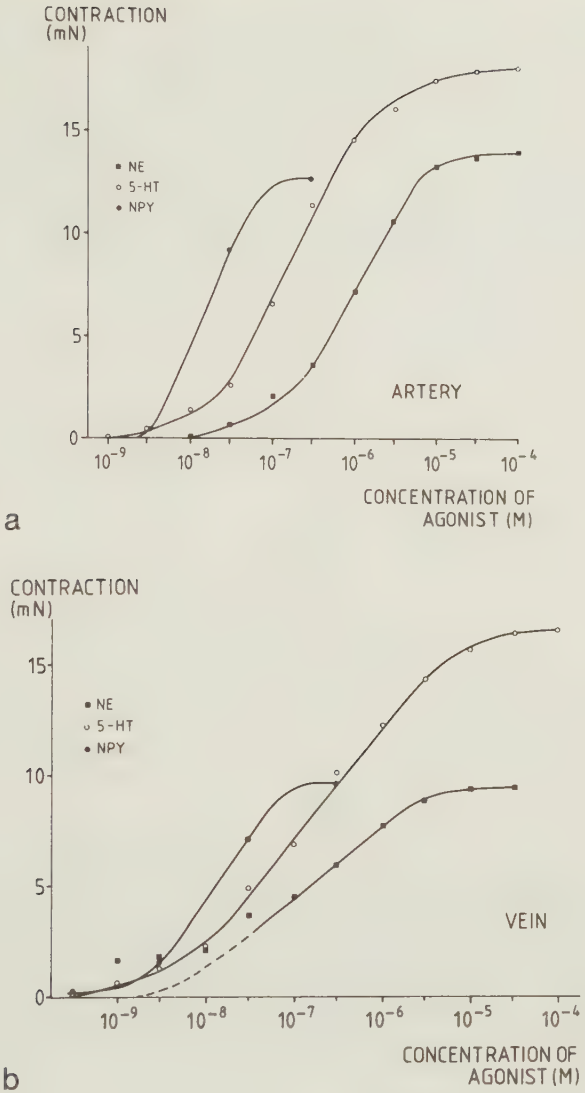


Fig. 2. Contractile response to norepinephrine (NE; n_{artery} = 22, n_{vein} = 27), 5-hydroxytryptamine (5-HT; n_{artery} = 30, n_{vein} = 25), and neuropeptide Y (NPY; n_{artery} = 6, n_{vein} = 7) in mesenteric arteries (a) and veins (b).

unity ($p > 0.05$; Fig. 3). The pA_2 value was 8.5 for phentolamine and 8.8 for prazosin, and the K_B values were $(1.9 \pm 0.9) \times 10^{-8}$ M and $(1.02 \pm 0.2) \times 10^{-9}$ M, respectively. Yohimbine (10^{-8}, 3×10^{-8} or 10^{-7} M), piperoxan (up to 10^{-6} M), ketanserin (10^{-9} to 10^{-7} M; IV) were without effect on the contractile response produced by NE. Clonidine (up to 10^{-3} M) was without contractile effect in 14 of 18 vessels tested. Oxymetazoline regularly contracted the arteries, but the range of E_{Am} was wide. The contractile action of 3×10^{-7} M oxymetazoline ($\sim EC_{50}$) was antagonized by 10^{-7} M prazosin, but not by the same dose of yohimbine.

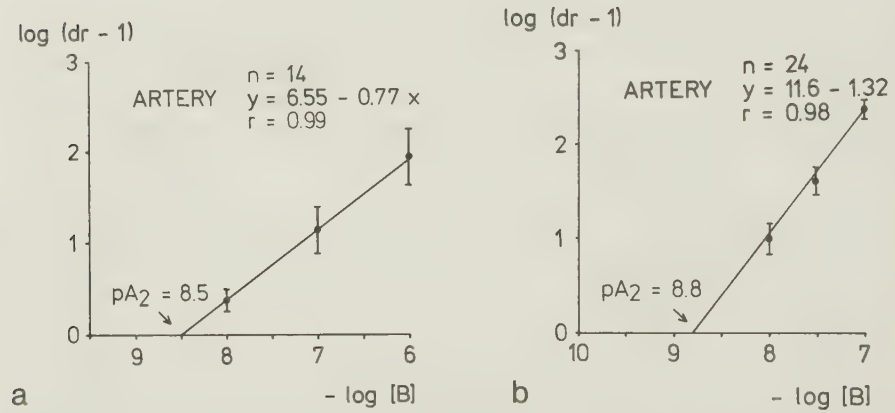


Fig. 3. Interaction between (a) norepinephrine and the α-receptor antagonist, phentolamine, and between (b) norepinephrine and the α_1-receptor antagonist, prazosin, in mesenteric arteries demonstrated in Schild plots. Number of curve displacements, equations for the lines, together with the correlation coefficients (r) are given; dr = dose ratio; [B] = agonist concentration; pA_2 = intercept of regression line with the abscissa.

α-receptor mediated contraction in mesenteric veins (paper III)

The potency rank for the sympathomimetic contraction of veins differed from that of arteries: epinephrine > clonidine ≥ norepinephrine = phenylephrine ≥ oxymetazoline > metoxamine > isoproterenol. Ratios indicated by equality signs were not statistically significant ($p > 0.05$) (Fig. 2b). Phentolamine, yohimbine and piperoxan inhibited the NE-induced contraction in a competitive manner. The true competitive antagonism was confirmed in Schild plots (Fig. 4). The pA_2 values were 8.6 (phentolamine), 8.3 (yohimbine),

and 7.6 (piperoxan). The K_B values were for phentolamine (7.7 $\pm$ 2.5) x 10^{-9} M, for yohimbine (3.8 $\pm$ 0.4) x 10^{-9} M and for piperoxan (5.3 $\pm$ 1.0) x 10^{-8} M. Prazosin (10^{-9} to 10^{-7} M), ketanserin (10^{-9} to 10^{-6} M), and R 55 667 (10^{-9} to 10^{-7} M) did not affect the concentration-response curve for NE. Clonidine contracted 6 of 7 veins, though the E_{Am} value varied. Both 3 x 10^{-6} M clonidine (~EC_{50}) and 3 x 10^{-7} M oxymetazoline (~EC_{50}) were inhibited by 10^{-7} M yohimbine, but not by the same dose of prazosin.

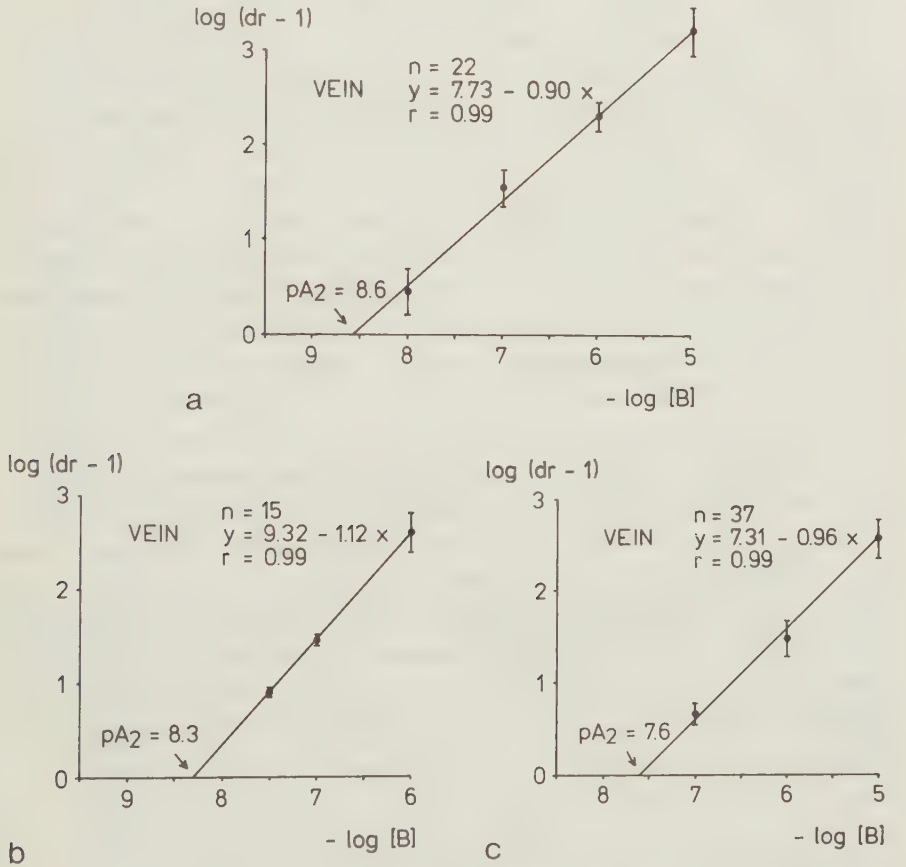


Fig. 4. Interaction between (a) norepinephrine and the α-receptor antagonist, phentolamine; between norepinephrine and the α_2-receptor antagonists yohimbine (b) and piperoxan (c), in mesenteric veins demonstrated in Schilds plots. Number of curve displacement, equations for the lines, together with the correlation coefficients (r) are given; dr = dose ratio; [B] = agonist concentration; pA_2 = intercept of regression line with the abscissa.

Dilatory response by amines in mesenteric vessels (papers III and IV)

Neither isoproterenol nor terbutaline or 5-HT, tested in doses 10^{-9} to 10^{-3} M, dilated arteries and veins precontracted with $PGF_{2\alpha}$. Both 10^{-10} M SP and 10^{-11} M carbacholine dilated the vessels.

Contractile response of 5-HT in mesenteric vessels (paper IV)

5-HT induced concentration-dependent contraction in both arteries and veins (Fig. 1, 5a and c). In the arteries the EC_{50} value, (2.5 $\pm$ 0.5) x 10^{-7} M, was lower ($p < 0.001$) than that obtained with NE (III), while the E_{Am} value (18.1 $\pm$ 3.6 mN) was the same as for NE. The veins, on the other hand, had an EC_{50}-value (5.6 $\pm$ 1.7) x 10^{-7} M similar to that with NE, but a higher E_{Am} value (16.4 $\pm$ 3.1 mN; $p < 0.05$). Ketanserin caused a dextral shift of the concentration-response curves for 5-HT. However, for both arteries and veins, the curves were deformed at antagonist concentrations above 3 x 10^{-8} M, so that only agonist concentrations higher than the EC_{50} levels were included in the parallel shift of the curves (Fig. 5a and c). Schild plots performed on the parallel parts of the curves gave lines with slopes not significantly different from unity ($p > 0.05$; Fig. 5b and d). The pA_2 values were 8.5 and 8.2 for arteries and veins, respectively, and the corresponding K_B values were (9.2 $\pm$ 3.0) x 10^{-9} M and (1.3 $\pm$ 5.4) x 10^{-8} M. There was also a tendency to reduced slope in the concentration-response curves, particularly at low 5-HT levels tested in the presence of high ketanserin concentrations. This effect was clearly distinguishable from a tachyphylaxic effect (Fig. 6). R 55 667 did not affect the EC_{50} values for 5-HT, but there was a progressive reduction in the E_{Am} values in both arteries and veins. For both ketanserin and R 55 667 the effect on the reduced E_{Am} values was mainly irreversible. Phentolamine in increasing concentrations reduced the E_{Am} values for 5-HT in both arteries and veins with an inconsistent effect on the EC_{50} values so that Schild plots could not be made.

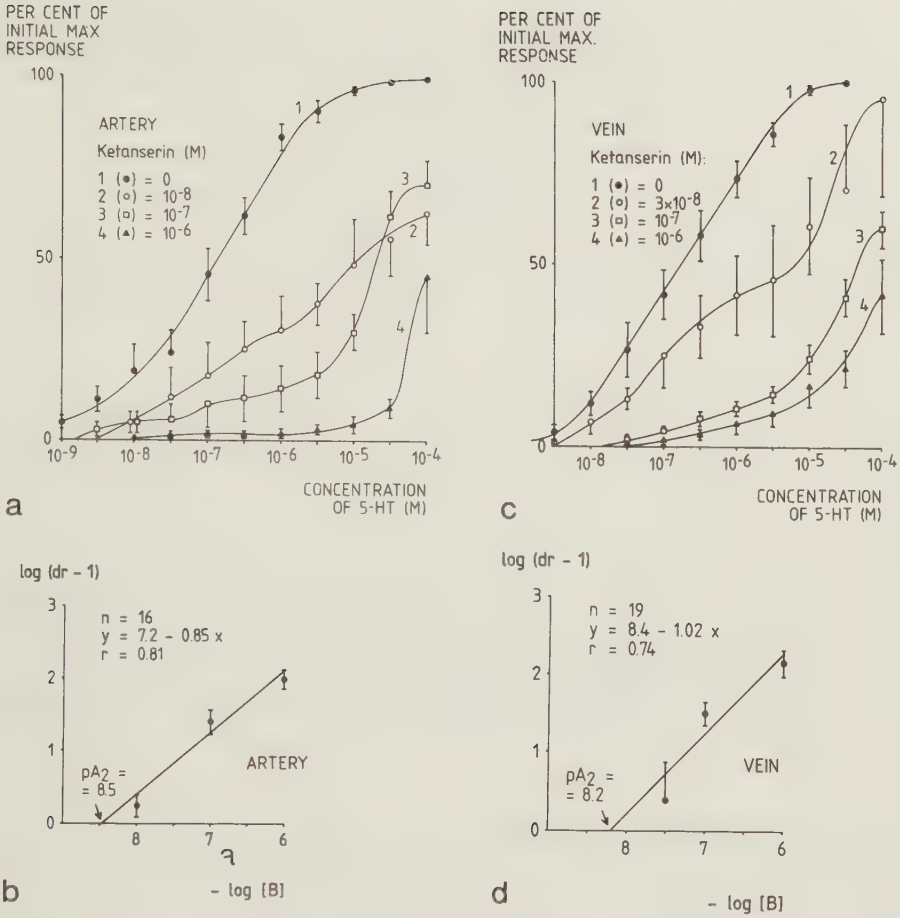


Fig. 5. Interaction between the serotonergic antagonist, ketanserin, and 5-HT in mesenteric vessels. (a) Arteries. Heterogenous inhibition (see text) of the concentration-response curves with increasing antagonist concentrations. Values are mean ± SEM of 3-14 vessels. The initial maximum response was set at 100 per cent, and the subsequent values obtained were related to this initial response. (b) Arteries. Schild plot based on 16 curve displacements (only components above EC_{50} used for calculation; see text) tested on 12 arteries. (c) Veins. Heterogenous inhibition of the concentration-response curves with increasing antagonist concentrations. Values are mean ± SEM of 4-14 vessels. The initial maximum response was set at 100 per cent, and the subsequent values obtained were related to this initial response. (d) Veins. Schild plot based on upper portions of 19 curve displacements tested on 12 veins. The equation for the lines are given in (b) and (d), together with the correlation coefficients (r); dr = dose ratio; [B] = agonist concentration; pA_2 = intercept of regression line with the abscissa.

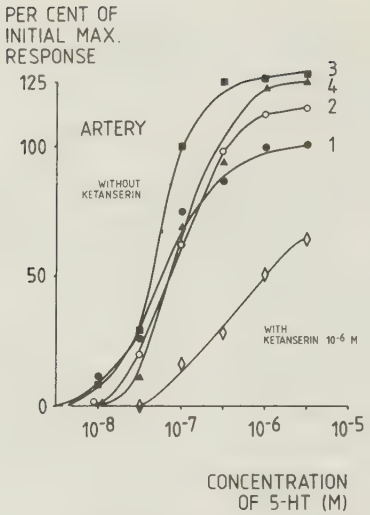


Fig. 6. Repeated full concentration-response curves to 5-HT alone (curves 1 to 4) or in the presence of 10^{-6} M ketanserin. Contractile response is expressed as per cent of the first test, each value being the mean of 4 segments from the same mesenteric artery. There was no significant changes in either E_{Am} or EC_{50} that could be attributed to tachyphylaxis, whereas ketanserin significantly inhibited the contraction.

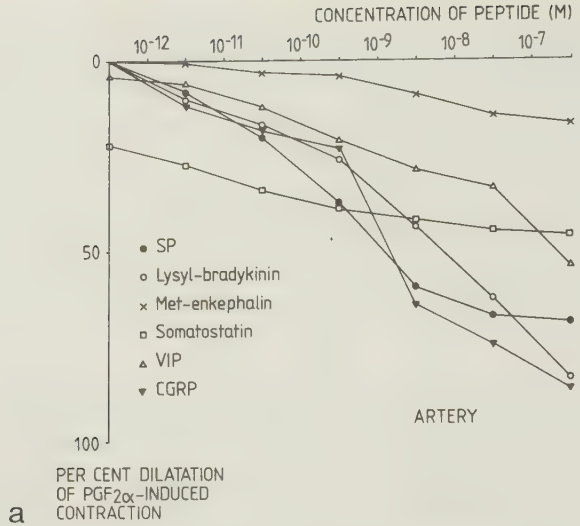
Effects of neuropeptides on vasomotor tone in mesenteric vessels (paper V)

Contractile effect. NPY caused a concentration-dependent contraction of both arteries (EC_{50} = (3.1 $\pm$ 0.1) x 10^{-8} M; E_{Am} = 12.9 $\pm$ 2.6 mN) and veins (EC_{50} = (1.8 $\pm$ 0.6) x 10^{-8}; E_{Am} = 9.8 $\pm$ 1.8 mN)(Fig. 2). In both types of vessels the EC_{50} values were lower than those obtained by NE (III), but the E_{Am} values were similar. However, both the EC_{50} and E_{Am} values varied considerably between different vascular preparations and sometimes even in the same patient. Neurotension gave a weak, but concentration-dependent contraction, in one of 13 arterial preparations, while no contraction was registered in the veins. Somatostatin was without contractile effect in the arteries, but did sometimes contract the veins in a inconsistent and unequivocal concentration-dependent way. SP, VIP, met- and leu-enkephalin, CGRP, and bradykinin were without contractile effect in both types of vessels, tested in doses up to 10^{-4} M.

Dilatory effect in arteries (Fig. 7a). Bradykinin, SP, VIP, somatostatin, and CGRP regularly dilated the arteries in a concentration-dependent way. The intrinsic dilatory activity of these five peptides (comparison of the EC_{50}-values) were in the order somatostatin $\geq$ SP = VIP $\geq$ CGRP $\geq$ bradykinin. The EC_{50} value for somatostatin was difficult to calculate, due to a non-sigmoidal shape of the concentration-response curve. The E_{Am} values were in the order: CGRP $\geq$ bradykinin $\geq$ SP $\geq$ VIP $\geq$ somatostatin. Met-enkephalin sometimes slightly dilated the arteries (2 of 23 preparations), but leu-enkephalin, neurotension as well as NPY was without dilatory effect.

Dilatory effect in veins (Fig. 7b). Bradykinin, SP and CGRP regularly caused vasodilatation with E_{Am} values similar to one another and to those obtained for the same peptides in the arteries. Also the EC_{50} values resembled those in the arteries: SP $\geq$ CGRP $\geq$ bradykinin. VIP dilated 9 of 15 veins with an intrinsic activity similar to that for CGRP and a potency as for bradykinin, SP, and CGRP.

As in arteries, a slight and inconsistent (2 of 15 preparations) dilatory effect was obtained with met-enkephalin. Leu-enkephalin, somatostatin, NPY, and neurotensin did not dilate the veins.



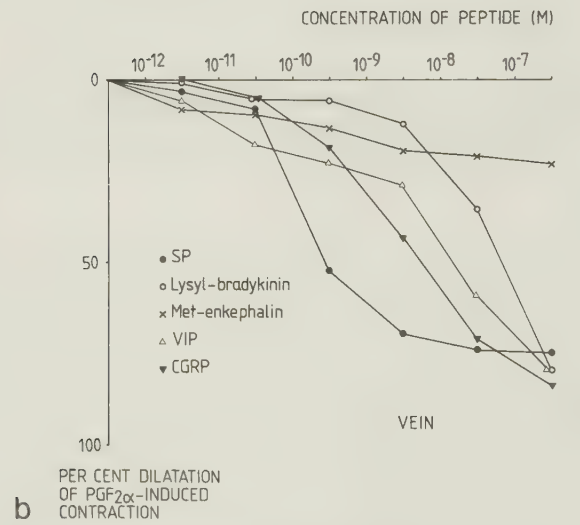


Fig. 7. (a) Dilatory response to substance P (SP) (●; n = 20), lysyl-bradykinin (o; n = 8), met-enkephalin (x; n = 2), somatostatin (□; n = 6), VIP (△; n = 12), calcitonin-gene related peptide (CGRP) (▼; n = 6) in mesenteric arteries precontracted with $PGF_{2\alpha}$ 10^{-6} M.

(b) Dilatory response to substance P (SP) (●; n = 9), lysyl-bradykinin (o; n = 7), met-enkephalin (x; n = 2), VIP (△; n = 5), and CGRP (▼; n = 7) in mesenteric veins precontracted with $PGF_{2\alpha}$ 10^{-6} M.

Inhibition of SP-induced dilatation in arteries and veins. The dilatory effect of SP was inhibited by Spantide in both arteries and veins (Fig. 8). However, the inhibition only caused a tendency to a dextral shift of the concentration-response curve at low antagonist concentrations and Schild plots could not be made.

Interaction between agonists.

5-HT versus NE (IV). Both 5-HT and NE, tested with doses near their EC_{50} values, markedly potentiated each other's contractile effect in human mesenteric arteries, but not in veins (Fig. 9).

Interaction between NPY and NE (VI). Various amounts of NPY (5×10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} M) did not cause any consistent change in the concentration-response curve for NE. Quantitative evaluation with 10^{-8} M NPY revealed no significant changes in the pD_2 values for NE in various human and animal vessels tested.

Presynaptic interaction between NPY and NE (VI). In electrically induced neurogenic vasoconstriction NPY potentiated the vasoconstriction more than 10 % in a concentration-dependent manner in 74 % of the experiments performed on rabbit auricular (n = 64), gastroepiploic arteries (n = 3), and human mesenteric arteries (n = 2) and veins (n = 5) (Fig. 10).

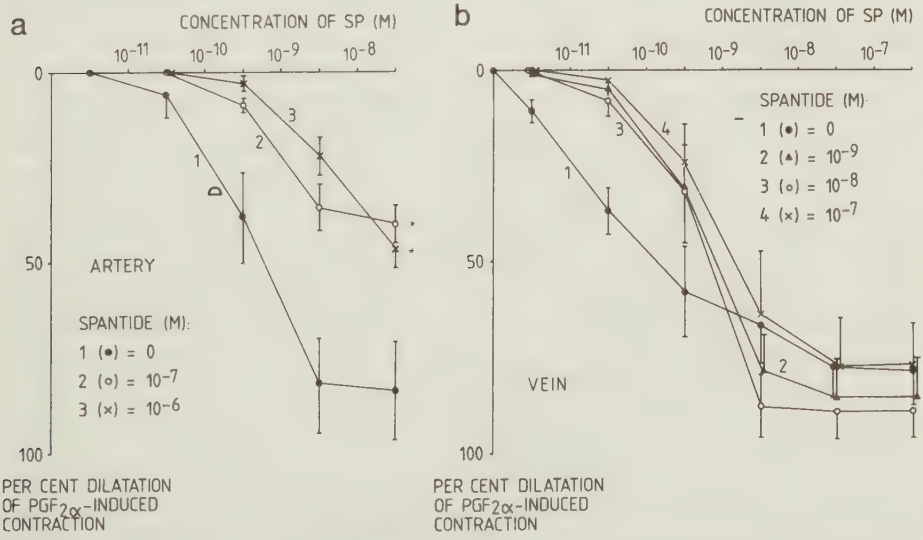


Fig. 8. Interaction between the substance P (SP)-antagonist ($D\text{-}Arg^1$, $D\text{-}Trp^{7,9}$, Leu^{11})SP, Spantide, and SP in precontracted (10^{-6} M $PGF_{2\alpha}$) mesenteric arteries (a) and veins (b). Values are mean + SEM of 4-7 arteries and 4-9 veins. Differences from values without antagonist: * $0.05 > p > 0.01$.

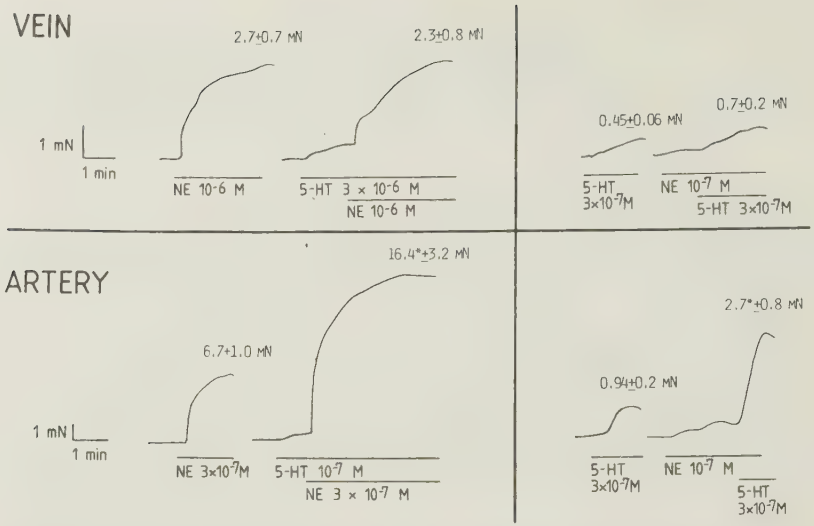


Fig. 9. Interaction between 5-HT and NE in mesenteric vessels. Typical examples are shown together with mean values ± SEM of 7 arteries and 8 veins. In the arteries, but not in the veins, both 5-HT and NE markedly potentiated each other's contractile actions.

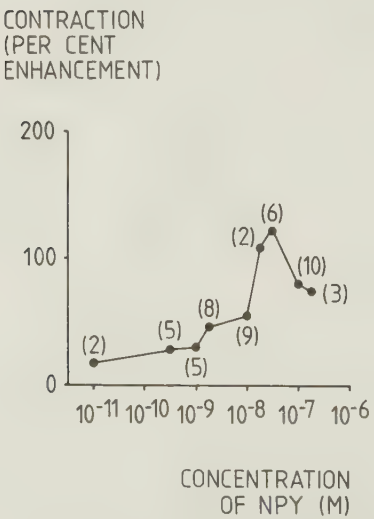


Fig. 10. The mean contraction induced by electrical field stimulation of rabbit's auricular arteries was potentiated in a concentration-dependent manner by NPY until a maximum effect was reached at a concentration of 5 x 10^{-8} M. Number of experiments within parenthesis.

DISCUSSION

In the clinical part of the present study, special interest has been focused on patients with malignant and advanced state of carcinoid tumours. All our patients had liver metastases, and it is known that the median survival time is about 24 months after the metastases have been diagnosed (Moertel et al., 1961). Most of our patients had pronounced increases in the blood and plasma levels of 5-HT and SP. Thus, according to earlier reports these patients should have been difficult to manage during anaesthesia (Stone and Donelly, 1960; Jones, 1962; Malavoud et al., 1970; Dery, 1971; Mason and Steane, 1976; Kleine et al., 1977; Desmonts et al., 1978; Jones and Knight, 1982) and a high incidence of carcinoid heart disease could have been expected, which also would have been an important lethal factor (Thorson et al., 1958; Roberts and Sjoerdsma, 1964; Trell et al., 1973; Costello, 1975; Callahan et al., 1982; Howard et al., 1982; Forman et al., 1984).

Our cardiological investigation revealed a low incidence of carcinoid heart involvement (19 %), which was in contrast to earlier reports (Callahan et al., 1982; Howard et al., 1982; Forman et al., 1984) in which right-sided valve lesions were observed in 43-57 % of the patients. This discrepancy can be explained by patient selection, in that a majority of the patients in other materials had been referred to cardiologic examination due to clinical signs of heart disease. In those materials also all patients without clinical signs of valve lesions had a normal echocardiogram which agrees with our findings. The relative ratio between pulmonic stenosis and tricuspid regurgitation in our study was similar to that reported by other workers (Roberts and Sjoerdsma, 1964; Trell et al., 1973), although a predominance of pulmonic stenosis has been found in some earlier reports (e.g., Thorson et al., 1954). The heart lesions have been attributed to high levels of 5-HT (Thorson et al., 1954; Roberts and Sjoerdsma, 1964; Trell et al., 1973), but we did not find any correlation between the measured high levels of blood 5-HT or SP and the degree of heart involvement. This is in accordance with the failure to produce heart lesions in rats by a chronic infusion of 5-HT (Waldenström, 1968). It has also been speculated that bradykinin through its ability to increase extravasation is responsible for the heart lesions (Jager and Polk, 1977), but the pathophysiology is still not clarified and probably there is a different sensitivity between the patients.

We have found a mild pulmonary hypertension in four (4/39 = 13 %) of our patients. This low incidence is surprising, considering the registered high levels of plasma 5-HT and its striking effect on pulmonary artery pressure found in animal experiments (Rudolph and Paul, 1957; Wanner et al., 1978). The same low incidence has been reported by Charms et al. (1959) and it was explained by a protective effect of right-sided heart lesions. Another speculative explanation could be the ability of SP to cause a release of histamine from mast cells (Johnson and Erdös, 1970; Erjavec et al., 1981), which in turn can mediate a relaxation of the pulmonary arteries via specific H_2 receptors (Boe et al., 1980; Mikkelsen et al., 1984) or H_1 receptors (Mikkelsen et al., 1984).

For the in vitro experiments in the present thesis mainly human blood vessels have been used, since a receptor variation has been described between species (Ruffolo et al., 1982). The mesenteric vessels were chosen since the splanchnic vascular bed receives as much as 25 % of cardiac output (Folkow and Neil, 1971), and thus is of significant hemodynamic importance. The aim was to obtain vessels from the terminal branches close to the intestines. In order to achieve intact preparations it was not possible to prepare human vessels with a diameter of less than 0.5 mm. Although resistance to flow is not primarily controlled by this large caliber vessels (Folkow and Neil, 1971) their pharmacological response seems to be representative of that exhibited by arteries with a diameter down to 0.1 mm (e.g. MacKenzie et al., 1978). For practical reasons the vessels were used after a storage time of up to 48 h. This time did not alter the dose-response reaction for either NE or 5-HT.

Several different techniques (Moulds, 1983) have been applied for studying motor responses of isolated blood vessels. Spiral strip preparations suffer from the disadvantage of damaging the musculature, nerves, and endothelium which are all cut when the strips are prepared. Whole-mounted ring preparations of vascular segments better reflect physiological conditions than the spiral strips because the morphology of the vessel wall remains intact and they primarily measure circular smooth muscle activity. We have used ring preparations (Nielsen and Owman, 1971; Edvinsson et al., 1974). The relationship between initial tension and force of contraction has been studied in various types of blood vessels (Edvinsson et al., 1974; Högestätt et al., 1981). Characteristically, there is a optimum in the level of initial tension at which maximal contraction is developed. The mesenteric vessels

in our study were given an amount of passive load, according to experiments with initial tension, that was well within the estimated maximum wall tension in vivo. Cumulative application of the agonists (van Rossum and van den Brink, 1963) was used. Different agonists were not tested on the same vascular segment, since it has been shown that prior contraction can result in altered responsiveness and maximum contractility (Rapoport and Bevan, 1983). For the same reason the vessels were not tested with potassium as standard. For testing the contractile response it was possible to carry out repeated runs for test periods up to 5 h. During that period the EC_{50} value remained stable though the E_{Am} value sometimes decreased. The decrease in E_{Am} was usually less than the variations in response for an individual vessel. A more pronounced decrease in maximum contractility was irregularly seen for 5-HT. This could be due to tachyphylaxis or to a declining response in an aging, partly damaged vessel. For the dilatory experiments the achieved active contraction remained steady for at least 30 min in most cases, and if there was a sagging in the curve, this was either taken into consideration when calculations were done or the experiment was excluded.

Recently it has become evident that the endothelial cells to a very high degree are functionally involved in the vascular response. Since Furchgott (1981) established that dilatation by acetylcholine, ADP, ATP, SP, and bradykinin are dependent on an intact endothelium which can release an endothelium-derived relaxing factor, this dependence has been confirmed by other workers and also shown to include thrombin, histamine, and 5-HT (Cohen et al., 1983; Vanhoutte and De Mey 1983; Furchgott, 1984; Furchgott et al., 1984). On the other hand, agents such as isoproterenol and nitrates do not depend on the endothelium for their effect (Furchgott et al., 1981; Vanhoutte et al., 1981). Another aspect on the role of the endothelium in vascular reactions has been presented by Cocks and Angus (1983). They showed that NE and 5-HT were significantly more powerful vasoconstrictors in the absence of endothelium. This has been found also by other workers for NE (Verrechia et al., 1984). Thus the endothelium can modulate the constrictory effect of amines, which must be considered in the response to vasoconstrictors in both normal and diseased vessels. - In our study both carbacholine and SP dilated the vessels at low concentrations. This indicates that the mounting in the organ bath did not damage the endothelium significantly. Thus, failure to elicit dilatation in our in vitro system may not simply be due to technical problems leading to endothelium damage.

In neither mesenteric arteries nor veins we were able to demonstrate any dilatory postjunctional β-receptors responsive to the agonists tested. The order of potency by which the classical sympathomimetic amines (Ahlquist, 1948) induced a constriction of the vascular preparations showed that the response involved α-adrenoceptors (NE > epinephrine > isoprenaline) (Furchgott, 1972). The relative potency of other sympathomimetic agonists gave, however, a more obscure picture when used to further classify the receptors into α_1 and α_2 subtypes. It is a general experience that subclassification of receptors only on the bases of agonist responses may be inappropriate. The relative potencies of agonists often agree with their relative affinity, provided the efficacies are equal (Starke, 1981). However, there is no obligatory relationship between affinity and efficiency (Ruffolo et al., 1980 a,b) and in our material there was a considerable variation in the E_{Am} values.

The results from the blocking experiments revealed a difference between mesenteric arteries and veins: in arteries the α_1 subtype predominates and in the veins the α_2 subtype is most common. A mixed population of α_1- and α_2-receptor can, as judged from the relative potencies of the agonists, not be excluded. This has also been seen in human post-mortem digital arteries and metacarpal veins (Stevens and Moulds, 1981). The situation thus resembles observations made on human arteries both from femoral and groin regions (Glusa and Markwardt, 1983) as well as from the omentum (Steen et al., 1983). Our conclusion is made since prazosin, which has a high affinity for the α_1-receptor and little or no affinity for the α_2-receptors (Cambridge and Greengrass, 1980), blocked the NE-induced contractile response in a competitive way without significant effect on the E_{Am} in the arteries, but was without effect in the veins. On the other hand, in the veins the α_2-antagonists, yohimbine and piperoxan, both inhibited the NE-induced contraction in a competitive way with unaltered E_{Am} values but did not affect the NE-response in the arteries. Piperoxan has a lower degree of selectivity for the α-receptor, but a preference for the α_2 subtype (cf. Borowski et al., 1977; Timmermanns et al., 1980, Doxey et al., 1983).

The pA_2 value (8.8) obtained with prazosin in the mesenteric arteries agreed well with corresponding values reported (8.2 - 8.9) for a variety of smooth muscle tissues (for references, see Skärby et al., 1983). In the veins yohimbine gave a pA_2 value (8.3) best corresponding to α_2- recep-

tors. Earlier reports have found corresponding values of 7.4-8.3 for the α_2-receptor, and 6.1-6.6 for the α_1-receptor (for references, see Skärby et al, 1983). The pA_2 value obtained (7.6) for yohimbine in the veins was in accordance with its interaction in other smooth muscle tissues (Timmermanns et al., 1980; Doxey et al., 1983).

In both mesenteric arteries and veins 5-HT induced a concentration-dependent contraction. According to the EC_{50} values, the intrinsic activity was similar in both types of vessels and comparable with human digital and intracranial arteries and veins (Edvinsson et al., 1978, 1984c; Arneklo-Nobin and Owman, 1985). The intrinsic activity in the arteries was higher for 5-HT than for NE.

The contractile response to 5-HT in mesenteric vessels seems to be mediated by 5-HT receptors since ketanserin, but not the α-blocker phentolamine, inhibited the contraction in a competitive way. This competitive inhibition corroborates previous observations (van Nueten et al., 1981; Arneklo-Nobin and Owman, 1985). Ketanserin is regarded to have a high selectivity for 5-HT_2 binding sites at low concentrations (van Nueten and Vanhoutte, 1984), which is also supported by receptor binding studies (Leysen et al., 1981). In both arteries and veins the inhibition of ketanserin caused a deformation of the concentration-response curves below EC_{50} values, but a parallel shift was achieved at higher 5-HT concentrations, although the E_{Am} values were lowered. Thus a component of competitive irreversible blockade must be involved. This may be a corresponding type of blockade as seen by phenoxybenzamine and other β-haloalkylamines of α-adrenoceptors (Furchgott, 1966). A depression of E_{Am} has also been found in canine venous smooth muscle (van Nueten et al., 1981).

We have also tested a newly introduced 5-HT_2 receptor blocker (R 55 667, Ritanserin) which has been proposed to have a higher affinity for the 5-HT_2 receptor and less α-receptor antagonistic action (Janssen, 1984). In our experiments the pronounced blockade of the 5-HT induced contraction appeared to be mainly non-competitive in both arteries and veins. The effect of Ritanserin in vitro reaches 75 % of its maximal effect after 15 min and 100 % after 30 min, and the effect persists unchanged for at least 2 h in contrast to Ketanserin which has a shorter onset and duration (Janssen, 1984). In our experiments, Ritanserin was added to the bath 10-15 min before the agonist, which thus can not explain the lack of competitive

inhibition of the 5-HT induced contraction. We did not find any α-blocking effects of either Ketanserin, or Ritanserin. This supports our conlusion that 5-HT in human mesenteric vessels acts on specific receptors and not on α-adrenoceptors, though others have found evidence that 5-HT acts on α-adrenoceptors, e.g. in the ear artery (Apperley et al., 1976; Fozard, 1976; Purdy, 1981).

5-HT has also been shown to be a potent vasodilator, mainly based on experiments in the intact organism (McCubbin et al., 1962; Walsh, 1967; McGrath et al., 1977; MacKenzie et al., 1978; Mecca and Webb, 1984), but also in isolated vascular preparations (Edvinsson et al., 1978). However, we were not able to demonstrate any 5-HT induced dilatation in mesenteric arteries or veins.

Of the different neuropeptides tested, only NPY regularly induced vasoconstriction in mesenteric vessels. The intrinsic activity of NPY was about 10 times higher than that we found for NE and 5-HT, while the potencies for these substances were similar. These results are in agreement with the vasoconstrictory and hypertensive effect following intravenous administration of NPY (Lundberg and Tatemoto, 1982) but somewhat contradictory to the results of Edvinsson and collegues (1984a) who reported that NPY induced only weak vasoconstriction in peripheral arteries of rabbits. The effect of NPY in our study was not influenced by tetrodotoxin, phentolamine, or Ketanserin, implicating an effect on smooth muscle receptors others than α- or 5-HT_2 receptors.

Many of the neuropeptides tested were potent vasodilators. The dilatation was less common in the veins, but this was probably not due to a lower inherent ability of the veins to dilate, since SP constantly induced the same relative response in both arteries and veins. The vasodilatation induced by SP is probably mediated by specific SP-receptors (Rosell et al., 1983b) located on endothelial cells (Furchgott, 1981). The assumption of the existence of specific receptors is based on experiments with SP-analogues which acts as SP-antagonists. We were not, however, able to pharmacologically classify the SP-receptor in the mesenteric vessels, since the inhibition of the SP-induced dilatation with Spantide (D-Arg^1, D-$Trp^{7,9}$, Leu^{11})SP was not purely competitive. The histamine-releasing properties of the SP-analogues (Håkanson et al., 1982) may have contributed to this lack of purely competitive inhibition.

Carcinoid tumours are known to contain not only 5-HT and SP but also bradykinin activating enzymes (Oates et al., 1964; Smith and Zeitlin, 1966). Part of the hypotensive reactions seen in carcinoid patients can be explained by the potent vasodilatory actions we found for bradykinin. This vasodilatation can thus be suspected to be additative to the effects exerted by SP, or in fact via SP (Butler et al., 1981).

In our patients with widespread carcinoid disease including liver metastases no remarkable changes in circulation or respiration during anaesthesia were observed, although the patients had a up to 1000-fold increase in 5-HT concentration in plasma. This is surprising considering that these patients have been regarded as 'high risk anaesthesia' (see Background). The explaination we propose is that our anaesthetic technique together with the close monitoring eliminate a considerable part of the problems. We used droperidol together with fentanyl. Droperidol gives a slight postsynaptic α-receptor blockade (Hyatt et al., 1980) and may thus prevent hypertension during intubation (Curran et al., 1980). Also fentanyl depresses stress and hypertension during laryngoscopy (Dahlgren and Messeter, 1981; Magnusson et al., 1983). The α-blockade of droperidol may induce hypotension, why the use of droperidol has been questioned (Mason and Steane, 1976), but this we have avoided by giving adequate amounts of fluids. This would give a dilated, well filled circulation with lower risk of dramatic hypotension induced by sudden hormone release from the tumour cells. We will particularly stress the importance of avoiding a hypovolemic situation, relative or absolute, during different phases of the peri-operative periods. This conviction is shared by Kleine and collegues (1977). We are also convinced that the close monitoring contributed to a safe anaesthetic procedure and the lack of major complications in the post-operative period in the present series. Early corrections can prevent a continuous hypotension with sympathetic stimulation which causes further release of vasoactive substances from the tumour cells (Levin and Sjoerdsma, 1963).

Pre-operative blockade of 5-HT receptors has been suggested to be of value in carcinoid patients (Dery, 1971), although the lack of specific blockers has hampered this approach (Kellermeyer and Graham, 1968; Graham-Smith, 1968; Mason and Steane, 1976). Our results with a non-specific 5-HT blocker, levopromazine (Mason and Steane, 1976), were not encouraging. The

patients were more vasodilated in the periphery, but during periods of hormone release (i.e. flushing), the heart work was more increased than in patients without this blockade.

A potentiating effect of the NE induced contraction by NPY has been claimed (Edvinsson et al., 1984 a,b; Ekblad et al., 1984b). We were not able to demonstrate any functionally significant potentiation on the postjunctional level, but NPY seemed to cause a concentration-related potentiation of the vasoconstrictor nerves. It was found that NE and 5-HT mutually potentiated each other's contractile effects at a postjunctional level in the human mesenteric arteries, but not in the veins, which is accordance with the results of other workers (de la Lande et al., 1966; Scroop and Walsh, 1968; van Nueten et al., 1981; Moreland and Bohr, 1983; Seabrook and Nolan, 1983). These potentiating effects, together with the earlier described 'inhibitory feedback loop' (Majewski and Starke, 1984), indicate that there is a complex interacting system between nerve terminals and effector cells. This may explain why blockade of the effects of only one substance, may disturb the balance on the effector cells. This phenomenon might be illustrated by the unfavourable effects of levopromazine in the carcinoid patients.

CONCLUDING REMARKS

Release of hormones peri-operatively in patients with metastatic carcinoids may lead to severe circulatory and respiratory disturbances. The present study has demonstrated that a modified neurolept anaesthesia without non-specific receptor blockade and with careful monitoring provides a safe procedure for carcinoid patients. Peri-operative changes in circulation and respiration did not correlate with changes in plasma 5-HT levels.

Heart involvement was found in about 20 % of our patients with advanced carcinoid disease. Slight pulmonary hypertension was established in 13 % of the patients. Carcinoid heart disease was in no patient a significant lethal factor.

Our pharmacological characterization of the vascular receptors in the isolated human mesenteric arteries and veins demonstrated that the contractile response to various sympathomimetic amines involved α-adrenoceptors. Postjunctionally the α_1 subtype of receptor predominated in the arteries, whereas the α_2 subtype was mainly found in the veins. 5-HT also caused contractions of the mesenteric vessels. The arteries were more sensitive to 5-HT than to NE, whereas in the veins the sensitivity to 5-HT and NE was similar.

The 5-HT_2 receptor antagonist, Ketanserin, caused a competitive partly irreversible inhibition of the 5-HT induced contraction.

NPY contracted both arteries and veins with an intrinsic activity about 10 times higher than that found for NE and 5-HT. CGRP, bradykinin, and SP regularly dilated both arteries and veins. The SP-antagonist, Spantide, interacted with the SP-induced dilatation in a not purely competitive way.

In low concentrations both 5-HT and NE potenciated each other's contractile effects in the arteries, but not in the veins. NPY potentiated the effect of NE during sympathetic nerve activation primarily through a presynaptic effect.

ACKNOWLEDGEMENTS

This thesis could not have been accomplished without the enthusiastic help and cooperation of many wonderful people. To name but a few:

Professor emeritus Eric Nilsson, who together with Professor Dick Thomson introduced me to this fascinating subject.

Professor Dag Lundberg, who has encouraged me to continue this line of research and followed it with great interest through its various phases.

My tutors and personal friends Associate Professor Anders Nobin and Professor Christer Owman who have shared their knowledge with me and were always ready to answer my many questions and did not dispair.

I would also like to thank Inga-Lill Bertilsson whose constant nagging I could not have done without. Thanks to her skilful assistance the practical work could be completed.

Thanks are due to those collegues in the departments of Anaesthesia, Histology, Cardiology, and Surgery who found time and had patience to help me.

All the secretaries of the different departments who were willing to do the sometimes endless typing and retyping.

Frans Boomsma who saved me in exile.

Above all these my sincere gratitude goes to Margareta for bearing with me.

This thesis was supported financially by grants from the Swedish Medical Research Council (grants No. 12X-712 and 14X-732) and from Janssen Leo Pharma AB.

REFERENCES

Ahlquist RP: A study of the adrenotropic receptors. Am J Physiol 153: 586-600 (1948).

Aluments J, Sundler F, Falkmer S, Ljungberg O, Håkanson R, Mårtensson H, Nobin A, Larsson Å: Neurohormonal peptides in endocrine tumors of the pancreas, stomach, and upper small intestine: I. An immunohistochemical study of 27 cases. Ultrastruct Pathol 5: 55-72 (1983).

Apperley E, Humphrey PPA, Levy GP: Receptors for 5-hydroxyptamine and noradrenaline in rabbit isolated ear artery and aorta. Br J Pharmacol 58: 211-221 (1976).

Arneklo-Nobin B, Owman Ch: Adrenergic and serotonergic mechanisms in human hand arteries and veins studied by fluorescence histochemistry and in vitro pharmacology. Blood Vessels 22: 1-22 (1985).

Arunlakshana O, Schild HO: Some quantitative uses of drug antagonists. Br J Pharmacol 14: 48-58 (1959).

Boe J, Boe M-A, Simonsson, BG: A dual action of histamine on isolated human pulmonary arteries. Respiration 40: 117-122 (1980).

Borowski E, Starke K, Ehrl H, Endo T: A comparison of pre- and postsynaptic effects of α-adrenolytic drugs in the pulmonary artery of the rabbit. Neuroscience 2: 285-296 (1977).

Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I: Calcitonin gene-related peptide is a potent vasodilator. Nature 313: 54-56 (1985).

Buccino RA, Covell JW, Sonnenblick EH, Braunwald E: Effects of serotonin on the contractile state of the myocardium. Am J Physiol 213: 483-486 (1967).

Butler JM, Cole DF, Zhang SW: Effect of trigeminal denervation on in vitro responses of rabbit iris sphincter muscle to bradykinin and substance P. J Physiol (London) 316: 18P-19P (1981).

Callahan JA, Wroblewski EM, Reeder GS, Edwards WD, Seward JB, Tajik AJ: Echocardiographic features of carcinoid heart disease. Am J Cardiol 50: 762-768 (1982).

Carlsson C: Karcinoidsyndromets anestesiologiska betydelse. Läkartidningen 69: 3657-3666 (1972).

Cambridge DD, Greengrass PM: The pharmacology of antihypertensive drugs with special reference to vasodilators, α-adrenergic blocking agents and prazosin. Prog Pharmacol 3/4: 107-114 (1980).

Charms BL, Kohn P, Applebaum HI, Geller J: Hemodynamic studies in a case of carcinoid cardiovascular syndrome. Circulation 20: 208-214 (1959).

Cocks TM, Angus JA: Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature 305: 627-630 (1983).

Cohen RA, Shepherd JT, Vanhoutte PM: 5-hydroxytryptamine can mediate endothelium-dependent relaxation of coronary arteries. Am J Physiol 245 (Heart Circ Physiol 14): H1077-1080 (1983).

Costa M, Furness JB, Cuello AC, Verhofstad AAJ, Steinbusch HWJ, Elde RP: Neurons with 5-hydroxytryptamine-like immunoreactivity in the enteric nervous system; their visualization and reactions to drug treatment. Neuroscience 7: 351-363 (1982).

Costello C: Carcinoid tumor metastases - prospective study of twenty-two patients. Am J Surg 130: 756-759 (1975).

Curran J, Crowley M, O'Sullivan G: Droperidol and endothracheal intubation. Attenuation of pressor response to laryngoscopy and intubation. Anaesthesia 35: 290-294 (1980).

Dahlgren N, Messeter K: Treatment of stress response to laryngoscopy and intubation with fentanyl. Anaesthesia 36: 1022-1026 (1981).

Dahlström A, Fuxe J: Evidence for the existence of monoamine neurons in the central nervous system. Acta Physiol Scand, Vol. 64, suppl 247: 7-85 (1965).

Déry R: Theoretical and clinical considerations in anaesthesia for secreting carcinoid tumors. Can Anaesth Soc J 18: 245-263 (1971).

Desmonts J-M, De Ribier A, Gilles M-R, Vic Dupont P: L'anesthésie et la rénimation dans la chirugie des carcinoides digistifs secrétants. Anesth Analg (Paris) 35: 143-153 (1978).

Douglas WW: Histamine and 5-hydroxytryptamine (serotonin) and their antagonists; in Goodman, Gilman, The pharmacological basis of therapeutics, McMillan, New York 1980, pp. 609-646.

Doxey JC, Roach AG, Smith CFC: Studies on RX 781094: a selective, potent and specific antagonist of α_2-adrenoceptors. Br J Pharmacol 78: 489-505 (1983).

Edvinsson L, Nielsen KC, Owman Ch: Influence of initial tension and changes in sensitivity during amine-induced contractions of pial arteries in vitro. Archs Int Pharmacodyn Thér 208: 235-242 (1974).

Edvinsson L, Hardebo JE, Owman Ch: Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man. Circ Res 42: 143-151 (1978).

Edvinsson L, Emson P, McCulloch J, Tatemoto K, Uddman R: Neuropeptide Y: Cerebrovascular innervation and vasomotor effects in the cat. Neurosci Lett 43: 79-84 (1983).

Edvinsson L, Ekblad E, Håkanson R, Wahlestedt C: Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. Br J Pharmacol 83: 519-525 (1984a).

Edvinsson L, Emson P, McCulloch J, Tatemoto K, Uddman R: Neuropeptide Y: Immunocytochemical localization to and effect upon feline pial arteries and veins in vitro and in situ. Acta Pysiol Scand 122: 155-163 (1984b).

Edvinsson L, Birath E, Uddman R, Lee TJ-F, Duverger D, MacKenzie ET, Scatton B: Indoleaminergic mechanisms in brain vessels; localization, concentration, uptake and in vitro responses of 5-hydroxytryptamine. Acta Physiol Scand 121: 291-299 (1984c).

Ekblad E, Håkanson R, Sundler F: VIP and PHI coexist with an NPY-like peptide in intramural neurones of the small intestine. Regul Pept 10: 47-55 (1984a).

Ekblad E, Edvinsson L, Wahlestedt C, Uddman R, Håkanson R, Sundler F: Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibres. Regul Pept 8: 225-235 (1984b).

Eklund B, Jogestrand T, Pernow B. Effect of substance P on resistance and capacitance vessels in the human forearm; in von Euler, Pernow, Substance P, Raven Press, New York 1977, pp. 275-285.

Erjavec F, Lembeck F, Florjanc-Irman T, Skofitsch G, Donnerer J, Saria A, Holzer P: Release of histamine by substance P. Naunyn-Schmiedebergs Arch Pharmacol 317: 67-70 (1981).

Emson PC, Gilbert RFT, Mårtensson H, Nobin A: Elevated concentrations of substance P and 5-HT in plasma in patients with carcinoid tumors. Cancer 54: 715-718 (1984).

Essman WB: Serotonin in health and disease. Vol 5, Spectrum, New York 1977-1978.

Euler US von, Gaddum JH: An unidentified depressor substance in certain tissue extracts. J Physiol (London) 72: 74-87 (1931).

Euler US von: The nature of adrenergic nerve mediators. Pharmac Rev 3: 247-277 (1951).

Fisher L, Kikkawa D, Rivier J, Amara S, Evans R, Rosenfeld M, Vale W, Brown M: Stimulation of noradrenic sympathetic outflow by calcitonin gene-related peptide. Nature 305: 534-535 (1983).

Folkow B, Neil E: Circulation. Oxford University Press, London 1971.

Forman MB, Byrd BF, Oates JA, Robertson RM: Two-dimensional echocardiogaphy in the diagnosis of carcinoid heart disease. Am Heart J 107: 492-496 (1984).

Fozard JR: Comparative effects of four migraine prophylactic drugs on an isolated extracranial artery. Eur J Pharmacol 36: 127-139 (1976).

Frank O: Die Elastizität der Blutgefässe. Z Biol 71: 255-272 (1920).

Furchgott RF: The use of β-haloalkylamines in the differentiation of receptors and in the determination of dissocation constants of receptor-agonist complex. Adv Drug Res 3: 21-55 (1966)

Furchgott RF: The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory; in Blaschko, Muscholl, Handbook of experimental pharmacology, Springer, Berlin 1972, pp. 283-335.

Furchgott RF: The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. Trends pharmacol Sci 2: 173-176 (1981).

Furchgott RF, Zawadzki JV, Cherry PD: Role of endothelium in the vasodilator response to acetylcholine; in Vanhoutte, Leusen, Vasodilation, Raven Press, New York 1981, pp. 49-66.

Furchgott RF: Role of endothelium in responses of vascular smooth muscle to drugs. Annu Rev Pharmacol Toxicol 24: 175-197 (1984).

Furchgott RF, Jothiananden D, Cherry PD: Endothelium dependent responses: The last three years. Biblthca cardiol, No. 38, Karger, Basel 1984, pp. 1-15.

Furness JB, Costa M, Emson PC, Håkanson R, Moghimzadeh E, Sundler F, Taylor IL, Chance RE: Distribution, pathways and reactions to drug treatment of nerves with neuropeptide Y- and pancreatic polypeptide-like immunoreactivity in the guinea-pig digestive tract. Cell Tissue Res 234: 71-92 (1983).

Glusa E, Markwardt F: Characterization of postjunctional α-adrenoceptors in isolated human femoral veins and arteries. Naunyn-Schmiedbergs Arch Pharmacol 323: 101-105 (1983).

Graham-Smith DG: The carcinoid syndrome. Am J Cardiol 21: 376-387 (1968).

Griffith SG, Burnstock G: Immunohistochemical demonstration of serotonin in nerves supplying human cerebral and mesenteric blood-vessels. Lancet 12: 561-562 (1983).

Hanko J, Hardebo J-E, Kåhrström J, Owman Ch, Sundler F: Calcitonin gene-related peptide (CGRP) is present in cerebrovascular nerve fibres and dilates pial and peripheral arteries. Neurosci Lett. In press. 1985a.

Hanko J, Törnebrandt K, Hardebo J-E, Kåhrström J, Nobin A, Owman Ch: Neuropeptide Y induces and modulates vasoconstriction in intracranial and peripheral vessels of animals and man. Submitted 1985b.

Hellstrand P, Järhult J: Effects of nine different gastrointestinal polypeptides on vascular smooth muscles in vitro. Acta Physiol Scand 110: 89-94 (1980).

Howard RJ, Drobac M, Rider WD, Keane TJ, Finlayson J, Silver MD, Wigle ED, Rakowski H: Carcinoid heart disease: Diagnosis by two-dimensional echocardiography: Circulation 66: 1059-1065 (1982).

Humphrey PPA: Pharmacological characterization of cardiovascular 5-hydroxytryptamine receptors; in Bevan et al., Vascular neuroeffector mechanisms, Raven Press, New York 1983, pp. 97-102.

Hyatt M, Muldoon SM, Rorie DK: Droperidol, a selective antagonist of postsynaptic α-adrenoceptors in the canine saphenous vein. Anesthesiology 53: 281-286 (1980).

Håkanson R, Bengmark S, Brodin E, Ingemansson S, Larsson L-I, Nilsson G, Sundler F: Substance P-like immunoreactivity in intestinal carcinoid tumors; in von Euler, Pernow, Substance P, Raven Press, New York 1977, pp. 55-58.

Högestätt ED, Andersson K-E, Edvinsson L: Mechanical reactivity of rat middle cerebral and basilar arteries to various vasoactive agents recorded in vitro; in Cervos-Navarro, Fritschka, Cerebral microcirculation and metabolism, Raven Press, New York 1981 pp. 181-187.

Högestätt ED, Andersson K-E, Edvinsson L: Mechanical properties of rat cerebral arteries as studied by a sensitive device for recording of mechanical activity in isolated small blood vessels. Acta Physiol Scand 117: 49-61 (1983).

Hökfelt T, Vincent S, Dalsgaard C-J, Skirboll L, Johansson O, Schultzberg M, Lundberg JM, Rosell S, Pernow B, Jansco G: Distribution of substance P in brain and periphery and its possible role as a co-transmitter. Ciba Foundation Symposium 91, Pitman, London 1982, pp. 84-106.

Iversen LL: The uptake and storage of noradrenaline in sympathetic nerves. Cambridge University Press, Cambridge 1967.

Jaffe BM: Prostaglandins and serotonin: non-peptide diarrheogenic hormones. World J Surg 3: 565-578 (1979).

Jager RM, Polk HC: Carcinoid apudomas; in Hickey, Current problems in cancer, Vol I. Chicago: Year Book Medical Publications Inc 1977: 11.

Janssen PM: R 55 667 - An original indolamine antagonist active in anxiety, tension states, and psychosomatic complaints. Investigational New Drug Brochure jan 1984, Janssen Pharmaceutica, n.v., B-2340 Beerse, Belgium.

Johnson AR, Erdös EG: Release of histamine from mast cells by vasoactive peptides. Proc Soc Exp Biol Med 142: 1252-1256 (1973).

Jones WPG: Serotonin release from carcinoid tumours. Can Anaesth Soc J 9: 42-50 (1962).

Jones RM, Knight D: Severe hypertension and flushing in a patient with a non-metastatic carcinoid tumour. Hypertension and flushing with a solitary carcinoid tumour. Anaesthesia 37: 57-59 (1982).

Jonson B, Nordström L, Olsson SG, Akerback D: Monitoring of ventilation and lung mechanics during automatic ventilation. A new device. Bull Physiopathol Resp 11: 729-743 (1975).

Katz J, Benumof J, Kadis LB: Anesthesia and uncommon diseases. Pathophysiologic and clinical correlations. Saunders, Philadelphia 1981.

Kellermeyer RW, Graham RC: Kinins - possible physiologic and pathologic roles in man. N Engl J Med 279: 754-759 (1968).

Kleine JW, Khouw YH, Heeres M: Management of anaesthesia during surgery for patients with carcinoid syndrome. Acta Anaesthesiol Belg 28: 165-170 (1977).

Koehler LS, Christides PS, Adamec TA: A complication of percutaneous cholangiography resulting in hypoxia and death of an anesthetized patient. Anesthesiology 49: 210-212 (1978).

De la Lande IS, Cannell VA, Waterson JG: The interaction of serotonin and noradrenaline on the perfused artery. Br J Pharmacol 28: 255-272 (1966).

Langer SZ, Shepperson NB: Subclassification of α-adrenoceptors into α_1- and α_2-categories: relevance to antihypertensive therapy; in Worcel, New trends in arterial hypertension. Inserm Symp. No. 17, Elsevier, North Holland Biomedical Press, Amsterdam 1981, pp. 73-85.

Langer SZ: Presynaptic regulation of the release of catecholamines. Pharmacol Rev 32: 337-362 (1981).

Larsson L, Edvinsson L, Fahrenkrug J, Håkanson R, Owman Ch, Schaffalitzky de Muckadell O, Sundler F: Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves. Brain Res 113: 400-404 (1976).

Leander S, Håkanson R, Rosell S, Folkers K, Sundler F, Tornqvist K: A specific substance P antagonist blocks smooth muscle contractions induced by non-cholinergic, non-adrenergic nerve stimulation. Nature (London) 294: 467-469 (1981).

Lembeck F, Holzer P: Substance P as a neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn-Schmiedebergs Arch Pharmacol 310: 175-183 (1979).

Levine RJ, Sjoerdsma A: Pressor amines and the carcinoid flush. Ann Int Med 58: 818-826 (1963).

Leysen JE, Awouters F, Kennis L, Laduron PM, Vandenberk J, Janssen PAJ: Receptor binding profile of R 41 468 a novel antagonist of 5-HT_2 receptors. Life Sci 28: 1115-1122 (1981).

Lieppe W, Behar VS, Scallion R, Kisslo JA: Detection of tricuspid regurgitation with two-dimensional echocardiography and periferal vein injections. Circulation 57: 128-132 (1978).

Lundberg JM, Änggård A, Fahrenkrug J, Hökfelt T, Mutt V: Vasoactive intestinal polypeptide (VIP) in cholinergic neurons of exocrine glands. Possible functional significance of co-existing transmitters for vasodilatation and secretion. Proc Natl Acad Sci USA 77: 1651-1655 (1980).

Lundberg JM, Tatemoto K: Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction reistant to -adrenoceptor blockade. Acta Physiol Scand 116: 393-402 (1982).

Lundberg JM, Terenius L, Hökfelt T, Martling C-R, Tatemoto K, Mutt V, Polak J, Bloom S, Goldstein M: Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. Acta Physiol Scand 116: 477-480 (1982).

Lundberg JM, Terenius L, Hökfelt T, Goldstein M: High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci Lett 42: 167-172 (1983).

Lundberg JM, Fahrenkrug J, Hökfelt T, Martling C-R, Larsson O, Tatemoto K, Änggård A: Co-existense of peptide HI (PHI) and VIP, in nerves regulating blood flow and bronchial smooth muscle tone in various mammals including man. Peptides 5: 593-606 (1984).

Macfarlane PS, Dalgliesh CE, Dutton RW, Lennox B, Nyhus LM, Smith AN: Endocrine aspects of argentaffinoma with special reference to the use of urinary 5-hydroxyindoleacetic acid estimations in diagnosis. Scott Med J 1: 148-155 (1956).

MacKenzie ET, Stewart M, Young AR, Harper AM: Cerebrovascular actions of serotonin: in Cervos-Navarro, Betz, Ebhardt, Ferszt, Wüllenweber, Pathology of cerebrospinal microcirculation, Raven Press, New York 1978 pp. 77-83.

Magnusson J, Werner O, Carlsson C, Nordén N, Pettersson K-I: Metoprolol, fentanyl and stress responses to microlaryngoscopy. Effects on arterial pressure, heart rate and plasma concentrations of catecholamines, ACTH, and cortisol. Br J Anaesth 55: 405-414 (1983).

Majewski H: Modulation of noradrenaline release through activation of presynaptic β-adrenoreceptors. J Auton Pharmacol 3: 47-60, corrigenda p.151 (1983).

Majewski H, Starke K: Prejunctional effects of catecholamines. Biblthca cadiol, No. 38, Karger, Basel 1984, pp. 53-59.

Malavaud A, Pedinielli L, Quilichini R, Sethian M: Problems anaesthésologiques posés par les carcinoids degestifs secrétants (a propos d'une observation). Anesth Analg (Paris) 27: 657-666 (1970).

Mason RA, Steane PA: Carcinoid syndrome: its relevance to the anaesthetist. Anaesthesia 31: 228-242 (1976).

McCubbin JW, Kaneko Y, Page IH: Inhibition of neurogenic vasoconstriction by serotonin. Circ Res 11: 74-83 (1962).

McGrath MA, Vanhoutte PM: Vasodilatation caused by peripheral inhibition of adrenergic neurotransmission; in Vanhoutte, Leusen, Mechanisms of Vasodilatation, Karger, Basel 1977, pp. 248-257.

Mecca TE, Webb RC: Serotonin and vasodilatation; in Vanhoutte, Vatner, Vasodilator mechanisms, Karger, Basel 1984, pp. 81-90.

Mikkelsen E, Sakr AM, Jespersen LT: Studies on the effect of histamine in isolated human pulmonary arteries and veins. Acta Pharmacol Toxicol (Kbh) 54: 86-93 (1984).

Miller R, Patel AU, Warner RRP, Parnes IH: Anaesthesia for the carcinoid syndrome: a report of nine cases. Can Anaesth Soc J 25: 240-244 (1978).

Miyatake K, Okamoto M, Kinoshita N, Ohta M, Kozuka T, Sakakibara H, Nimura Y: Evaluation of tricuspid regurgitation by pulsed doppler and two-dimensional echocardiography. Circulation 66: 777-784 (1982).

Moertel CG, Sauer WG, Dockerty MB, Bagenstoss AH: Life history of the carcinoid tumor of the small intestine. Cancer 14: 901-912 (1961).

Moreland RS, Bohr DF: Prior exposure to serotonin attenuates norepinephrine contraction in dog mesenteric artery. Blood Vessels 20: 203 (1983).

Moulds RFW: Techniques for testing isolated blood vessels. Gen Pharmacol 14: 47-53 (1983).

Mårtensson H, Nobin A, Sundler F: Carcinoid tumors in the gastrointestinal tract - an analysis of 156 cases. Acta Chir Scand 149: 607-616 (1983).

Mårtensson H, Nobin A, Sundler F; Falckmer S: Endocrine tumors of the ileum - cytochemical and clinical aspects. Pathol Res Pract. In press (1985).

Neglén P: Aortic clamping. Skeletal muscle metabolism and central circulation during abdominal reconstructive vascular surgery for arteriosclerotic disease. MD Thesis, Lund, Sweden (1980).

Nielsen KC, Owman Ch: Contractile response and amine receptor mechanisms in isolated middle cerebral artery of the cat. Brain Res 27: 33-42 (1971).

Nobin A, Karp W, Lunderquist A, Rosengren E, Sandén G, Sundler F: Localization of carcinoids and pheochromocytomas with vein catheterization and amine determination. Brain Res Bull 9: 781-797 (1982).

Nobin A, Björklund A: Topography of the monoamine neuron system in the human brain as revealed in fetuses. Acta Physiol Scand, suppl 388: 1-40 (1973).

van Nueten JM, Jansson PAJ, van Beek J, Xhonneux R, Verbeuren TJ, Vanhoutte PM: Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT_2 serotonergic receptors. J Pharmacol Exp Ther 218: 217-230 (1981).

van Nueten JM, Vanhoutte PM: Cardiovascular pharmacology of ketanserin. Biblthca cardiol, No. 38, Karger, Basel 1984, pp. 222-233.

Oates JA, Melmon K, Sjoerdsma A, Gillespie L, Mason DT: Release of a kinin peptide in the carcinoid syndrome. Lancet 1: 514-517 (1964).

Olsson SG, Fletcher R, Jonson B, Nordström L, Prakash O: Clinical studies of gas exchange during ventilatory support - a method using the Siemens-Elema CO_2 analyzer. Br J Anaesth 52: 491-499 (1980).

Owman, Ch: Vascular autonomic nerves and corresponding receptors, with particular reference to neurogenic mechanisms of vasodilatation. Prog Pharmacol 3/4: 47-68 (1980).

Owman Ch, Andersson J, Hanko J, Hardebo J-E: Neurotransmitter amines and peptides in the cerebrovascular bed; in MacKenzie, Seylaz, Bès, L.E.R.S. Monograph Series, Vol. 2: Neurotransmitters and the cerebral circulation, Raven Press, New York 1984, pp. 11-38.

Pagé IH, McCubbin JW: Variable arterial pressure response to serotonin in laboratory animals and man. Circ Res 1: 354-362 (1953).

Patel KD, Dalal FY: Anaesthetic management of a patient with carcinoid tumor undergoing myocardial revascularization. Can Anaesth Soc J 27: 260-263 (1980).

Pernow B: Substance P. Pharmacol Rev 35: 85-141 (1983).

Purdy RE, Hurlbut DE, Rains LA: Receptors for 5-hydroxytryptamine in rabbit isolated ear artery and aorta. Blood Vessels 18: 16-27 (1981).

Rapoport RM, Bevan JA: Effect of contraction on the subsequent responsiveness and maximum contractility of the rabbit ear artery and saphenous vein in vitro. Blood Vessels 20: 44-55 (1983).

Roberts WC, Sjoerdsma A: The cardiac disease associated with the carcinoid syndrome (carcinoid heart disease). Am J Med 36: 5-34 (1964).

Rosell S, Björkroth U, Xu J-C, Folkers K: The pharmacological profile of a substance P (SP) antagonist. Evidence for the existence of subpopulations of SP receptors. Acta Physiol Scand 117: 445-449 (1983a).

Rosell S, Björkroth U, Folkers K: Subpopulations of substance P (SP) receptors and separate eledoisin receptors; in Skrabanek, Powell, Substance P Dublin 1983, Boole Press, Dublin 1983b, pp. 61-62.

Rosenfeld MG, Mermod JJ, Amara SG, Swanson LW, Sawchenko PE, Rivier J, Vale WW, Evans RM: Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature 304: 129-135 (1983).

Rossum JM van, Brink FG van den: Cumulative dose-response curves. I. Introduction to the technique. Arch Int Pharmacodyn Thér 143: 240-246 (1963).

Rowell LB: Active neurogenic vasodilatation in man; in Vanhoutte, Leusen, Vasodilatation, Raven Press, New York 1981, pp. 1-17.

Rudolf AM, Paul MH: Pulmonary and systemic vascular response to continuous infusion of 5-hydroxytryptamine (serotonin) in the dog. Am J Physiol 189: 263-268 (1957).

Ruffolo RR, Wadell JE, Yaden EL: Receptor interactions of imidazolines. IV. Structural requirements for alpha-adrenergic receptor occupation and receptor activation by clonidine and a series of structural analogues in rat aorta. J Pharmacol Exp Ther 213: 267-272 (1980a).

Ruffolo RR, Yaden EL, Wadell JE: Receptor interactions of imidazolines. V. Clonidine differentiates postsynaptic alpha-adrenergic receptor subtypes in tissues from the rat. J Pharmacol Exp Ther 213: 557-561 (1980b).

Ruffolo RR, Waddell JE, Yaden EL: Heterogeneity of postsynaptic alpha-adrenergic receptors in mammalian aorta. J Pharmacol Exp Ther 221: 309-314 (1982).

Schild HO: The use of drug antagonists for identification and classification of drugs. Br J Pharmacol 2: 251-258 (1947).

Scroop GC, Walsh JA: Interactions between angiotensin, noradrenaline and serotonin on peripheral blood vessels in man. Aust J Exp Biol Med Sci 46: 573-580 (1968).

Seabrook JM, Nolan PL: The vascular interaction of noradrenaline and 5-hydroxytryptamine. Eur J Pharmacol 89: 131-135 (1983).

Shepherd JT, Vanhoutte PM: The human cardiovascular system. Raven Press, New York (1979).

Skärby T, Andersson K-E, Edvinsson L: Pharmacological characterization of isolated feline cerebral and peripheral arteries. Acta Physiol Scand 117: 63-73 (1983).

Smith AN, Zeitlin IJ: The role of bradykinin in vasomotor aspects of the carcinoid and dumping syndromes. Br J Surg 53: 867-869 (1966).

Starke K: Regulation of noradrenaline release by presynaptic receptor systems. Rev Physiol Biochem Pharmacol 77: 1-124 (1977).

Starke K: α-Adrenoceptor subclassification. Rev Physiol Biochem Pharmacol 88: 199-236 (1981).

Steen S, Skärby TVC, Norgren L, Andersson K-E: Pharmacological characterization of postjunctional α-adrenoceptors in isolated human omental arteries and veins. Acta Physiol Scand 120: 109-116 (1983).

Steinbusch HWM: Serotonin-immunoreactive neurons and their projections in the CNS; in Björklund, Hökfelt, Handbook of chemical neuroanatomy, Vol 3. Classical transmitters and transmitters receptors in the CNS, Part II, Elsevier, Amsterdam, New York, Oxford 1984, pp. 68-125.

Stevens MJ, Moulds RFW: Heterogeneity of postjunctional α-adrenoceptors in human vascular smooth muscle. Archs Int Pharmacodyn Thér 254: 43-47 (1981).

Stjernquist M, Emson P, Owman Ch, Sjöberg N-O, Sundler F, Tatemoto K: Neuropeptide Y in the female reproductive tract of the rat. Distribution of nerve fibres and motor effects. Neurosci Lett 39: 279-284 (1983).

Stone HH, Donnelly CC: The anesthetic significance of serotonin secreting carcinoid tumors. Anesthesiology 21: 203-212 (1960).

Strickman NE, Rossi PA, Massumkhani GA, Hall RJ: Carcinoid heart disease: A clinical pathologic and therapeutic update. Curr Probl Cardiol 6: 11-20 (1982).

Sundler F, Moghimzadeh E, Håkanson R, Ekelund M, Emson P: Nerve fibres in the gut and pancreas of the rat displaying neuropeptide Y immunoreactivity. Cell Tissue Res 230: 487-493 (1983).

Tatemoto K, Carlquist M, Mutt V: Neuropeptide Y - a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 296: 659-660 (1982).

Terenghi G, Polak JM, Allen JM, Zhang SQ, Unger WG, Bloom ST: Neuropeptide Y-immunoreactive nerves in the uvea of the guinea pig and rat. Neurosci Lett 42: 33-38 (1983).

Thorson Å, Biörck G, Björkman G, Waldenström J: Malignant carcinoid of the small intestine with metastases to the liver, valvular disease of the right side of the heart (pulmonary stenosis and tricuspid regurgitation without septal defects), peripheral vasomotor symptoms, bronchoconstriction, and an unusual type of cyanosis. Am Heart J 47: 795-817 (1954).

Thorson Å: Studies on carcinoid disease. Acta Med Scand, suppl, 334 (1958).

Timmermans PBMWM, Meel JCA van, Zwieten PA van: Evaluation of the selectivity of α-adrenoceptor blocking drugs for postsynaptic α_1- and α_2-adrenoceptors in a simple animal model. J Auton Pharmacol 1: 53-60 (1980).

Trell E, Rausing A, Ripa J, Torp A, Waldenström J: Carcinoid heart disease. Clinicopathologic findings and follow-up in 11 cases. Am J Med 54: 433-444 (1973).

Törnebrandt K, Nobin A, Ericsson M, Thomson D: Circulation, respiration, and serotonin levels in carcinoid patients during neurolept anaesthesia. Anaesthesia 38: 957-967 (1983).

Törnebrandt K, Nobin A, Owman Ch: Pharmacological characterization of alpha-adrenergic receptor subtypes mediating contraction in human mesenteric arteries and veins. Blood Vessels 22: 000-000 (1985).

Törnebrandt K, Eskilsson J, Nobin A: Heart involvement in metastatic carcinoid disease. Clin Cardiol 8: 000-000 (1985).

Törnebrandt K, Nobin A, Owman Ch: Serotonergic smooth muscle receptors in human mesenteric arteries and veins. Submitted 1985.

Törnebrandt K, Nobin A, Owman Ch: Vasomotor effects of nine neuropeptides on isolated human mesenteric blood vessels. Submitted 1985.

Uddman R, Alumets J, Edvinsson L, Håkanson R, Sundler F: VIP nerve fibres around peripheral blood vessels. Acta Physiol Scand 112: 65-70 (1981).

Uddman R, Sundler F, Emson P: Occurrence and distribution of neuropeptide Y-immunoreactive nerves in the respiratory tracts and middle ear. Cell Tissue Res 23: 321-327 (1984).

Uddman R, Ekblad E, Edvinsson L, Håkanson R, Sundler F: Neuropeptide Y-like immunoreactivity in perivascular nerve fibres of the guinea-pig. Reg Pept 10: 243-257 (1985).

Unger T, Rockhold RW, Yukimura T, Rettig R, Ganten D: Blood pressure and heart rate response to central iv administered substance P are increased in spontaneously hypertensive rats. Clin Sci 59: 299-302 (1980).

Vanhoutte PM: Heterogeneity of vascular smooth muscle; in Kaley, Alture. Microcirculation, Vol. II, University Park Press, Baltimore 1978, pp. 181-389.

Vanhoutte PM, Verbeuren TJ, Webb RC: Local modulation of adrenergic neuroeffector interaction in blood vessel wall. Physiol Rev 61: 151-247 (1981).

Vanhoutte PM, De Mey J: Control of vascular smooth muscle function by the endothelial cells. Gen Pharmacol 14: 39-41 (1983).

Vanhoutte PM, Shepherd JT: Muscarinic and β-adrenergic prejunctional modulation of adrenergic neurotransmission in the blood vessel wall. Gen Pharmacol 14: 35-37 (1983).

Vanhoutte PM: Serotonin and hypertension; in Bevan et al., Vascular neuroeffector mechanisms, Elsevier Publishers B.V., Amsterdam 1984, pp. 329-332.

Verrechia C, Sercombe R, Seylaz J: Vasoconstrictor responses influenced by the endothelium. Biblthca Cardiol, No. 38, Karger, Basel 1984, pp. 16-20.

Waldenström J: Clinical picture of carcinoidosis. Gastroenterology 54: 826-828 (1968).

Walsh JA: Antagonism of methysergide of vascular effects of 5-hydroxytryptamine in man. Br J Pharmacol 30: 518-530 (1967).

Wanner A, Begin R, Cohn M, Sackner MA: Vascular volumes of the pulmonary circulation in intact dogs. J Appl Physiol 44: 956-963 (1978).

Zinner MJ, Yeo CJ, Jaffe BM: The effect of carcinoid levels of serotonin and substance P on hemodynamics. Ann Surg 2: 197-204 (1984).

Anaesthesia, 1983, Volume 38, pages 957–967

Circulation, respiration and serotonin levels in carcinoid patients during neurolept anaesthesia

K. TÖRNEBRANDT, A. NOBIN, M. ERICSSON AND D. THOMSON

Summary

Release of hormones peri-operatively in patients with metastatic carcinoids may lead to severe circulatory and respiratory disturbances. Fourteen patients with liver metastases were studied during 16 operations with a modified neurolept anaesthesia in order to evaluate the central haemodynamic and respiratory functions as well as plasma serotonin levels. The premedication in five patients was supplemented with levopromazine. During the 11 operations performed on patients not pretreated with levopromazine, no major significant fluctuations in circulatory or respiratory functions were recorded although big variations in serotonin plasma levels were measured. In the patients treated with levopromazine, however, significant changes were observed in heart rate, mean pulmonary artery pressure, cardiac index, and left and right ventricular stroke work especially during flushing episodes. However, these changes did not correlate with the changes in plasma serotonin levels. Modified neurolept anaesthesia without levopromazine pretreatment combined with careful monitoring seems to be a safe procedure for carcinoid patients. Using this type of anaesthetic procedure only one major complication occurred in connexion with 16 major operations and then in the postoperative period.

Key words

Monitoring; blood pressure, cardiac output, peripheral vascular resistance.
Hormones; serotonin.

The incidence of carcinoids (endocrine tumours of the digestive tract) varies notably from one series to another, but this tumour probably constitutes about 1% of the malignant tumours in Sweden.[1] Fifty per cent of carcinoids emanate from the appendix and they rarely metastasize; the rest originate from elsewhere in the bowel and often metastasize to regional lymph nodes and the liver.[2,3] The carcinoids form and release a series of active substances[4–8] such as serotonin, bradykinin, prostaglandins and substance which are responsible for the symptoms seen in these patients, such as flushing, diarrhoea, headache, palpitations and wheezing (carcinoid syndrome).[7,9,10]

With more refined techniques for the diagnosis and localisation of carcinoid tumours[11] and with encouraging results after surgical intervention,[12] an increasing number of carcinoid patients undergo surgery. It is well recognised that these patients are difficult to manage during anaesthesia. Mason and Steane[13] reported that compli-

K. Törnebrandt, MD, Senior Registrar, Department of Anaesthesiology, A. Nobin, MD, PhD, M. Ericsson, MD, PhD, Consultants, Department of Surgery, D. Thomson, MD, PhD, Consultant, Department of Anaesthesiology, University Hospital of Lund, Lund, Sweden.

0003-2409/83/100957 + 11 $03.00/0 © 1983 The Association of Anaesthetists of Gt Britain and Ireland

cations including severe tachycardia, hyper- or hypotension and bronchiolar constriction occurred during surgery in more than 50% of the patients. Anaesthesia, surgical stress and manipulation of the tumour are said to precipitate these responses.[13–17]

Several anaesthetic techniques have been proposed for theoretical reasons or based only on case reports.[13–23] The advantages and disadvantages of these techniques are considered in the discussion. The aim of this study of carcinoid patients undergoing intestinal and liver surgery is to elucidate the frequency of disturbances in circulatory and respiratory functions with a standardized anaesthetic technique and analyse possible correlations between these disturbances and changes in plasma serotonin levels.

Materials and methods

Ten male and four female patients with carcinoid aged 36–71 years, all with liver metastases, were selected sequentially and were studied during 16 operations. They were all normotensive and, except in one patient who had a cardiac dysrhythmia, there was no history of cardiac disease. Patient data are presented in Table 1. All sixteen cases were premedicated with droperidol, 5 mg intramuscularly (i.m.), and fentanyl, 0.1 mg i.m., about 45 minutes before arrival in the operating room. Cases one to five received in addition pretreatment with levopromazine, 12.5 mg i.m., on the night before surgery and 12.5 mg i.m. at the time of premedication.[24] This routine with levopromazine pretreatment was abandoned, for reasons discussed below, in the remaining patients.

The patients were grouped separately according to whether they had received levopromazine or not when the results were evaluated. The study was performed with the informed consent of the patients and approved by the Ethical Committee of the University of Lund.

Anaesthesia

The patients were premedicated as above. Prior to induction an arterial and a triple lumen thermistor pulmonary artery catheter were inserted under local anaesthesia. The electrocardiogram (ECG) was continuously monitored. Anaesthesia was induced by inhalation of 50% nitrous oxide and oxygen 50%, and droperidol 0.15 mg/kg, together with fentanyl, 0.015 mg/kg, intravenously over a period of 5 minutes. Pancuronium, 0.1 mg/kg, was given to facilitate nasotracheal intubation which was performed after spraying with 80 mg lignocaine. All patients were ventilated using a volume controlled ventilator (Siemens-Elema, Servo 900B) with an end tidal carbon dioxide (CO_2) analyser 930.[25] The patients were ventilated with a mixture of 65% nitrous oxide and oxygen 35%, the minute volume being set to maintain an end tidal tension ($P_E'CO_2$) of about 4 kPa. Supplemental doses of fentanyl (0.5 mg/hour operating time in divided doses) and pancuronium were given as required during the operation.

Intravenous fluids were started with 1 litre of Ringer's lactate prior to the induction. After induction, maintenance fluid using 5.5% glucose with 80 mmols sodium/litre was given at a rate sufficient to give a urinary output of 1 ml/kg/hour and to maintain cardiac filling pressures to the normal range (central venous pressure (CVP)/ pulmonary capillary wedge pressure (PCW) 5/8 mmHg). Blood losses were replaced unit for unit.

Measurements

Cardiac output was determined at frequent intervals on a thermodilution computer (Edwards, model 9520) using 5.5% glucose injected at room temperature. Five measurements were made. The highest and lowest values were excluded and the mean value of the remaining three was taken. Airway pressures and flow as well as $P_E'CO_2$ and CO_2 production were monitored using the Servoventilator and, together with the circulatory variables, were registered on an eight-channel ink-writer (Mingograph 81, Siemens-Elema).

The calculated haemodynamic variables used were: mean arterial blood pressure (MAP, normal 70–105 mmHg); cardiac output (CO, normal 4–8 litres/minute); cardiac index (CI, normal 2.5–4.0 litre/minute/sq m body surface area); stroke volume (SV, normal 60–100 ml); mean pulmonary artery pressure (MPAP, normal 15–20 mmHg); pulmonary capillary wedge pressure (PCW, normal 6–12 mmHg); central venous pressure (CVP, normal 0–5 mmHg); systemic vascular resistance (SVR = (MAP–CVP) × (1/ CO), normal 10–15 mmHg/litre minute/sq m); left ventricular stroke work (LVSW = SV × (MAP–PCW) × 0.0136, normal 45–75 g m); pulmonary vascular resistance (PVR = (MPAP–PCW) ×

Table 1. Clinical data on 14 patients with carcinoid tumour and liver metastases at the time of 16 operations

	Age and sex	Site of primary tumour	Carcinoid syndrome	5-HIAA (μmol/24 hours urine)	Serotonin (ng/ml PPP)	Serotonin (ng/ml whole blood)	Previous operation	Present operation
1	51 M	Ileum	+	130	11	—	Small bowel resection	Temporary de-arterialization; cholecys-tectomy
2	56 M	Ileum	+	170	447	—	Small bowel resection	Temporary de-arterialization; cholecys-tectomy
3	36 M	Ileum	–	611	14	—	Exploratory laparotomy	Ileotrans-versostomy
4*	64 M	Ileum	–	69	3	—	Small bowel resection	Ileocaecal resection; debulking of liver metastasis
5	72 M	Ileum	+	178	14	—	Ileocaecal resection and debulking of liver metastasis	Temporary de-arterialization; cholecystectomy
6*	64 M	Ileum	–	32	27	129	Ileocaecal resection and debulking of liver metastasis	Temporary de-arterialization; cholecysectomy
7	59 M	Ileum	–	55	22	—	Small bowel resection	Exploratory laparotomy
8	54 F	Ileum	–	850	113	814	Small bowel resection	Exploratory laparotomy
9†	63 F	Ileum	+	94	38	425	Ileotrans-versostomy	Right hemicolectomy; small bowel resection
10	67 F	Ileum	+	2140	1200	975	—	Temporary de-arterialization; cholecystectomy
11	64 F	Caecum	+	330	106	919	Ileocaecal resection	Temporary de-arterialization
12	72 M	Ileum	+	128	85	495	Ileocaecal resection	Temporary de-arterialization; cholecystectomy
13	57 M	Ileum	+	53	3	247	Ileocaecal resection	Debulking of liver metastasis
14	40 M	Ileum	+	136	55	2041	Small bowel resection; debulking of liver metastasis	Temporary de-arterialization
15†	63 F	Ileum	+	58	7	269	Ileotrans-versostomy	Temporary de-arterialization; cholecystectomy
16	51 M	Ileum	+	660	60	1047	—	Temporary de-arterialization; cholecystectomy
Normal values, mean (SEM)				< 80	2.4 (1.2)	124.3 (12.6)		

M = male, F = female, PPP = platelet poor plasma fraction.
*Operations Nos 4 and 6 are the same patients. †Operations Nos 9 and 15 are the same patient.

(l/CO), normal < 3.12 mmHg/litre minute/sq m; right ventricular stroke work (RVSW = SV × (MPAP–CVP) × 0.0136, normal 10–15 g m). Formulae in Cardiopulmonary Analysis Programs for Hewlett Packard 67 and 97 Calculators, modified after Civetta, 1977 (see Neglén[26]).

The lung mechanical values, inspiratory resistance, expiratory resistance and compliance of the respiratory system were calculated according to formulae given by Johnson *et al.*[27]

Cardiac output was measured and haemodynamic variables were calculated before induction, before surgery, during a period of the operation when no or little surgical stimulation was applied ('during surgery'), and 'during flushing'.

Biochemical methods

For the analysis of serotonin, blood samples were collected at intervals before and during the operations from a catheter placed in the right hepatic vein. The catheter was inserted via the femoral vein on the morning of the day of operation and the position of the catheter was checked with fluoroscopy.

Analysis of serotonin was performed in a platelet-poor plasma fraction (PPP) and in whole blood. The number of platelets in our preparations averaged 10×10^9/litre plasma (normal platelet count $170–300 \times 10^9$/litre plasma). The serotonin was determined with liquid chromatography with electrochemical detection.[11]

Statistical methods

Statistical evaluation of the results was made by using Student's *t*-test for paired observations within patients and unpaired between groups. Spearman's rank coefficient was used for testing correlation. All tests were two-sided. Probability values of $p < 0.05$ were considered to indicate statistical significance.

Results

Haemodynamics

In all patients a decrease in mean arterial pressure (MAP) was seen after induction with return to pre-anaesthetic values after surgical incision. No other changes were seen either between those who were pretreated with levopromazine and those without pretreatment or between the two groups (Fig. 1).

After induction, heart rate (HR) fell in both

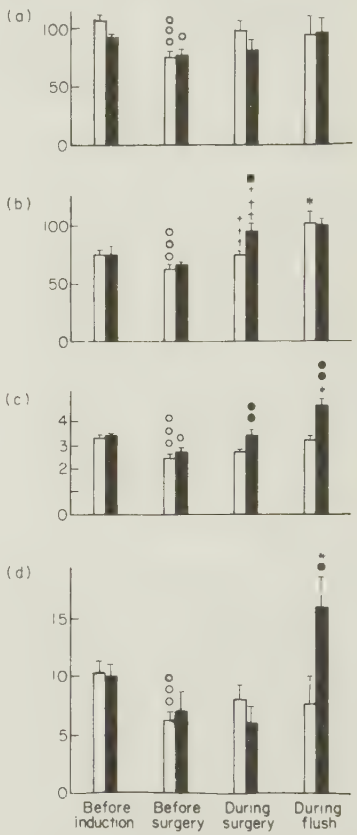


Fig. 1. (a) Mean arterial pressure (mmHg), (b) heart rate (beats/minute), (c) cardiac index (litres/minute/sq m body surface area) and (a) right ventricular stroke work (g m) in 16 patients with carcinoid before and during anaesthesia.

Values are means (SEM) of 11 determinations in patients not pretreated with levopromazine and five determinations in the pretreated patients (except for during flush when four determinations were made in each group). Differences from values within groups before induction: ○ $0.05 < p < 0.01$; ○○○ $p < 0.001$. Differences from values between groups during surgery and during flush: ● $0.05 < p < 0.01$; ●● $0.01 < p < 0.001$. Differences from values within groups before surgery: ††† $p < 0.001$. Differences from values within groups during surgery: * $0.05 < p < 0.01$. □, non-pretreated values; ■ levopromazine pretreated patients.

Table 2. Mean (SEM) values of cardiovascular variables in non-pretreated patients and levopromazine pretreated patients before induction, before surgery, during surgery and during flush

	Before induction		Before surgery		During surgery		During flush	
	Non-pretreated patients ($n = 11$)	Levopromazine pretreated patients ($n = 5$)	Non pretreated patients ($n = 11$)	Levopromazine pretreated patients ($n = 5$)	Non-pretreated patients ($n = 11$)	Levopromazine pretreated patients ($n = 5$)	Non-pretreated patients ($n = 4$)	Levopromazine pretreated patients ($n = 4$)
SVR (mmHg/litre min/sq. m)	191.2 (2.2)	14.8 (1.1)	17.5 (2.0)	15.25 (1.4)	21.4 (1.9)	13.0 (0.9)●	18.8 (3.5)	11.4 (2.5)
LVSW (g m)	106 (8.3)	106 (14.5)	63.0 (7.1)	75.2 (11.4)	79.6 (8.5)	70.8 (14.3)	71.5 (14.7)	108 (9.5)●*
PVR (mmHg/litre min/sq. m)	1.30 (0.2)	1.00 (0.08)	1.04 (0.07)	9.67 (0.16)	1.38 (0.33)	1.02 (0.06)	1.35 (0.15)	1.13 (0.24)
MPAP (mmHg)	13.1 (1.1)	10.8 (1.5)	11.9 (1.1)	10.2 (1.8)	14.1 (1.1)	9.8 (1.0)●	12.8 (1.8)	16.8 (1.9)*

Differences from values within groups before induction: $0.01 > p > 0.001$; ⁻ $p < 0.001$.
Differences from values between groups during surgery and during flush: ● $0.05 > p > 0.01$.
Differences from values within groups during surgery: * $0.05 > p > 0.01$.

groups, but this was significant only in the non-pretreated patients. Heart rate increased significantly in both groups after surgical incision and more significantly in the pretreated group. During flush this difference disappeared due to a greater increase in heart rate in the group without levopramazine pretreatment (Fig. 1).

Except for a lower mean pulmonary artery pressure (MPAP) during surgery with a significant increase during flush in the pretreated group, there were no differences from the stable values in the non-pretreated group (Table 2).

Cardiac index (CI) decreased significantly in both groups after induction. The group without pretreatment then remained stable but CI in the other patients with pretreatment increased after incision and became significantly higher. This increase was significantly larger during flush (Fig. 1).

Systemic and pulmonary vascular resistance (SVR and PVR) remained unchanged in both groups, but SVR was lower in the levopromazine pretreated group and this difference was significant during surgery (Fig. 1, Table 2).

Left and right ventricular stroke work (LVSW and RVSW) decreased in both groups after induction. This decrease was, however, not significant in RVSW for the pretreated group. LVSW and RVSW remained unchanged during surgery in both groups, but increased significantly during flush in the levopromazine group (Fig. 1, Table 2).

When the pulmonary artery catheter was withdrawn at the end of the operation, no significant pressure gradient indicating a pulmonary stenosis was found between the pulmonary artery and the right ventricle.

Respiration

The Pa_{O_2} and Pa_{CO_2} values remained normal during the whole procedure in all patients.

Only one patient (No. 16, Table 3) developed signs of bronchiolar constriction, with rapid fluctuations in the airway resistance unrelated to arterial blood pressure. The inspiratory resistance in this case was calculated to change from 1.8 to 2.7 kPa/litre/sec (normal 1.6, SD 4) and the expiratory resistance, at the start of expiration, from 2.0 to 9.0 kPa/litre/sec (normal 2.1, SD 7) over a period of 20 minutes. The changes in airway resistance in this patient, as represented by peak inspiratory and pause pressures, do not appear

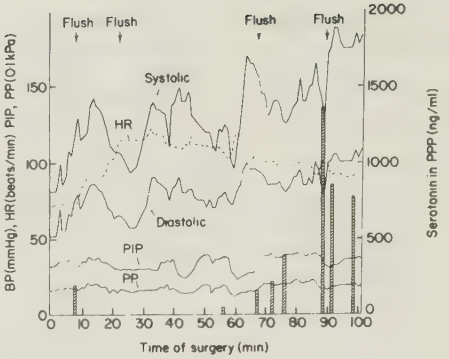


Fig. 2. In patient 16 the relationship during surgery between arterial blood pressure (BP), heart rate (HR), peak inspiratory pressure (PIP), pause pressure (PP) and serotonin in platelet-poor plasma (PPP). ⊠.

to be related to HR, blood pressure or plasma concentrations of serotonin (Fig. 2). As there was no effect on the blood gases, no treatment was considered necessary. There was no change in compliance in spite of the variations in the airway resistance. No rapid changes in the breath by breath CO_2 elimination were seen, indicating that the pulmonary circulation in this patient was relatively unchanged in spite of the fluctuations in systemic arterial blood pressure.

Metabolism

While all patients were normoglycaemic before the operation and had the same rate of dextrose infusion, six patients showed intra-operative hyperglycaemia which required short acting, insulin. Three of these patients also had metabolic acidosis necessitating sodium bicarbonate (Table 3). However, the continuously registered $P_{E'CO_2}$ and $\dot{V}CO_2$ production were stable, indicating no major changes in the overall metabolism demanding increased ventilation during the operations.

Serotonin concentrations in PPP and whole blood

Before induction of anaesthesia the concentration of serotonin in whole blood from ten patients ranged from 129 to 2041 ng/ml (mean 736 ng/ml (SEM 179) Table 1) and the concentrations in PPP for sixteen patients ranged from 3 to 1200

Table 3. Data during surgery

Operation	Hyperglycaemia (mmol/litre)	Base excess (mmol/litre)	Serotonin (ng/ml PPP)	Bronchiolar constriction	Number of flushes
1	—	—	10–172	—	2
2	—	—	159–474	—	8
3	—	—	11–148	—	—
4	—	—	2–11	—	2
5	—	—	6–314	—	1
6	—	—	8–55	—	—
7	—	—	0.7–22	—	1
8	18	—	14–152	—	—
9	—	—	5–38	—	—
10	20	—	246–3225	—	—
11	16	−9	53–548	—	3
12	17	−6.5	8–96	—	—
13	—	—	1–4	—	—
14	—	—	15–110	—	4
15	17	−8	2–15	—	—
16	31	−7	50–1360	+	5

ng/ml, mean 138 ng/ml PPP (SEM 76), Table 1).

Three patients (Nos 4, 13 and 15) had serotonin levels within or close to the normal range (Table 1); in this group two patients had experienced the carcinoid syndrome pre-operatively and one flushed during surgery.

In the group of 13 patients with pre-operatively elevated plasma serotonin concentrations, ten suffered from the carcinoid syndrome. The basal serotonin concentrations in PPP did not differ between patients with or without the carcinoid syndrome. Six patients with elevated serotonin levels flushed during surgery; all of these had a previous history of flushing. During intubation increases in serotonin concentrations were registered in four patients (Nos 5, 8, 14, 16) but this did not result in simultaneous changes in blood pressure and HR (Table 4). Manipulation of the carcinoid tissue resulted in a more than 100% increase in serotonin concentrations in PPP in six patients, while minor or no changes were recorded in eight patients. Flushing episodes were seen five times simultaneously with tumour manipulation but in only one case concomitant with an increase in serotonin concentration in plasma. Many flushing episodes were observed during surgery and, during 13 of these, serotonin measurements were performed. The 13 flushes occurred in eight patients of whom all but one (No. 4) had a pre-operative history of flushing. During flushing, the serotonin concentration in PPP increased more than 100% on five occasions and by 50% on one occasion (Table 4). Unchanged serotonin levels were noted in seven flushing episodes.

There was no statistical correlation between the serotonin concentrations or variations and the levels or variations in either HR, MAP or MPAP.

Awakening

Carcinoid patients are said to waken slowly after anaesthesia,[14] and only four patients could be extubated directly after surgery. Eleven patients were mechanically ventilated during the first 12 hours postoperatively. During this period no sedative drugs were needed. These patients were thereafter extubated without complications. One patient suffered from postoperative carcinoid shock and was extubated on the fifth postoperative day (see below).

Postoperative course

In fifteen patients, the immediate postoperative course was uneventful and the patients could leave the recovery room after about 12 hours. One patient (No. 10, Table 1) with an uneventful anaesthesia had a complicated postoperative course. This patient went into carcinoid shock with hypotension, oliguria, oedema and adult respiratory distress syndrome 12 hours after the operation and required symptomatic treatment

Table 4. Variations (Δ) in systolic blood pressure (SBP), heart rate (HR) and serotonin levels at different stages during 16 operations in carcinoid patients

	Intubation			Start of operation			Tumour manipulation			Flushing		
Operation	Δ SBP (mmHg)	Δ HR (/min)	Δ Serotonin (ng/ml PPP)	Δ SBP (mmHg)	Δ HR (/min)	Δ Serotonin (ng/ml PPP)	Δ SBP (mmHg)	Δ HR (/min)	Δ Serotonin (ng/ml PPP)	ΔSBP (mmHg)	ΔHR (/min)	ΔSerotonin (ng/ml PPP)
1	0	0	0	+95	0	0	−45	+50	+160	−35	0	+160
										−35	+60	not reg.
2	+30	+30	0	+50	0	0	−40	+10	0	−20	+20	0
										−60	+10	0
										−15	−5	+311
3	0	0	0	0	0	+38	0	0	+137	—	—	—
4	+20	+20	+3	+50	0	+5	+5	0	0	−20	0	0
5	+35	0	+265	+30	0	0	+15	+20	+194	−25	0	0
6	0	0	0	+20	+5	0	+25	+20	0	—	—	—
7	+30	−5	0	+50	+10	0	0	0	0	−10	+5	0
8	0	0	+38	+60	0	0	+50	+5	+138	—	—	—
9	0	0	0	+50	+10	0	−40	0	+13	—	—	—
10	0	0	0	+20	+15	0	+30	+15	+570	—	—	—
11	0	0	0	+30	+15	0	0	0	0	+20	+10	+46
										−65	+15	not reg.
12	+20	+25	0	+25	0	+17	0	0	0	—	—	—
13	+35	+15	0	+10	0	0	—	—	—	—	—	—
14	+70	0	+64	+25	0	+8	—	—	—	−25	0	0
										−45	+30	+5
										−40	+15	not reg.
15	0	0	0	+50	+10	0	−50	+15	+8	—	—	—
16	0	0	+15	+40	+10	0	+15	+5	+130	+15	−10	+130
										−20	−10	+107
										−40	+10	+970

for 3 days in the intensive care unit. The patient recovered without sequelae.

Discussion

This study has shown that there are significant variations in the cardiovascular function as well as in plasma serotonin concentration during anaesthesia and surgery of carcinoid patients, while changes in the respiratory function are rare. When using a modified neurolept anaesthesia technique combined with careful monitoring, the circulatory and respiratory deviations observed are easily managed and the clinical effects can be minimised. This finding contrasts with reports stating that these patients are difficult to manage during anaesthesia[13,14,18] due to hypo- or hypertension, tachycardia or bronchospasm refractory to treatment.

Pre-operative blockade of the serotonin receptors has been suggested to minimise complications.[14] At present, lack of specific blockers has, however, made this approach of minor significance.[13,28,29] The customary routine in this hospital of non-specific serotonin receptor blockage with levopromazine[24] was used in the first five patients. These patients were more vasodilated compared to the others, as reflected in a lower SVR. A low peripheral resistance during surgery may be of benefit but, on the other hand, levopromazine did not seem to abolish the hormonal effect on systemic and pulmonary circulation during flushing, because the work of the right and left ventricle increased. Routine levopromazine pretreatment is therefore now abandoned.

Several anaesthetic techniques have been proposed for carcinoid patients.[13–19,21,22] Regional anaesthesia is unsuitable because the liberated hormones act directly on the peripheral receptors and thus are not influenced by denervation.[14,16,17] On the other hand, the technique can be of some benefit in preventing release of catecholamines from the adrenal medulla.

Inhalation anaesthesia with, for example, halothane or enflurane is another possibility, but hypotension due to myocardial depression and peripheral vasodilation must be avoided as this can precipitate hormone release via stress mechanisms.[16] However, in a hypertensive state, this effect may be useful[23] if the volatile anaesthetic is used as a supplement.

In this study we have used neurolept anaesthesia with droperidol and fentanyl. Droperidol produces a slight postsynaptic α-receptor blockade[30] and may thus prevent hypertension during intubation.[31] Mason and Steane[13] have questioned the use of droperidol due to the risk of hypotension induced via the α-receptor blockade, but in our experience this hypotension can be avoided by giving adequate amounts of fluids prior to induction. The importance of avoiding hypovolaemia in these patients who often have a history of diarrhoea is also stressed by Kleine and his colleagues,[19] who claim that if the circulating blood volume is sufficient there is usually no severe protracted hypotension or shock. The long half life of droperidol should be no drawback since awakening is delayed anyway due to altered brain serotonin metabolism,[14] often necessitating postoperative mechanical ventilation. Fentanyl has been shown to possess antihistaminergic H_1 and antiserotoninergic actions.[32] A large initial dose and liberal maintenance doses should be given to prevent adrenergic overactivity during intubation and surgery. Morphine is not used since it can cause release of histamine or serotonin.[33] The intubation trauma may also be minimised by local anaesthetics.[15,16,22]

It is preferable to achieve neuromuscular block with pancuronium rather than with tubocurarine because the histamine release is considerably smaller.[13,34,35] Pancuronium may also have a constrictive effect on the capacitance vessels,[36] while tubocurarine is a ganglionic blocker. Suxamethonium was not used because induced muscle fasciculations and the resultant rise in intra-abdominal pressure can cause hormone release.[13,37] This precaution is of minor relevance since we demonstrated that even vigorous tumour manipulation does not reliably produce hormone release or obvious flushing.

Flushes are often associated with remarkable vasomotor phenomena such as hyper- or hypotension or tachycardia, and the flushing episodes have therefore been considered as dangerous during anaesthesia. The vasomotor changes are explained by extended release of active substances from the carcinoid tumour during the flush. Serotonin is a direct peripheral vasoconstrictor but also a peripheral inhibitor of neurogenic vasoconstriction.[38] In situations with a low sympathetic tone, as during anaesthesia, the vasopressor effect predominates. Bradykinins and substance P, which are supposed to be

responsible for the flushing are potent vasodilatators and may be responsible for the hypotension sometimes observed during the flushing period.[39] Preliminary observations in our patients also revealed an increase in the plasma substance P levels during surgery. It is noticeable that conspicuous alterations in haemodynamic function were lacking during flushing in the patients who had not been pretreated with levopromazine. This occurred despite serotonin levels fluctuating greatly during the flushing episodes in several patients. On the other hand pronounced changes in MPAP, CI, RVSW and LVSW were observed during flushing in the patients pretreated with levopromazine, which is regarded as a non-specific serotonin receptor blocker. An explanation can be that blockage of the receptor for only one of the active substances released during flush may interfere with the balance between the substances resulting in negative final effects.

Periods of bronchospasm in these patients could be expected due to the effects of serotonin and different peptides.[7,14] Considering the very high levels of serotonin (up to 3225 ng/ml PPP) in many of our patients, it is surprising that bronchospasm was seen only in one (patient no. 6). This absence of lung mechanical changes during flushing is at variance with the experience of others[14,29] and may be attributable to the modified neurolept anaesthesia.

Our results demonstrate that carcinoid patients usually have high basal levels of serotonin in PPP and that striking variations in plasma concentrations occur during operations on these patients. As serotonin is mainly bound to platelets, its measurement should be performed in a platelet-poor plasma fraction, thus making evaluation of small changes in the active plasma pool possible. By contrast, significant variations in serotonin concentrations in whole blood were rarely significant.

In conclusion, we would recommend this technique of modified neurolept anaesthesia with intensive haemodynamic monitoring since no adverse effects were seen from increased secretion of serotonin and other active substances even when a 1000-fold fluctuation in serotonin concentration was measured. Since the only complications occurred postoperatively, it is suggested that close monitoring is continued.

Acknowledgments

This study was supported by a grant from the Swedish Medical Research Council. The authors thank Mrs Karin Fyge, Miss Annika Hindemith and Miss Monica Keidser for skilful technical assistance.

References

1. Berge T, Linell F. Carcinoid tumours. Frequency in a defined population during a 12-year period. *Acta Pathologica et Microbiologica Scandinavica Section A* 1976; **84:** 322–30.
2. Moertel CG, Sauer WG, Dockerty MB, Baggenstoss AH. Life history of the carcinoid tumor of the small intestine. *Cancer* 1961; **14:** 901–12.
3. Mårtensson H, Nobin A, Sundler F. Carcinoid tumors in the gut. *Acta Chirurgica Scandinavica* 1983: in press.
4. Håkansson R, Bengmark S, Brodin E, Ingemansson S, Larsson L-I, Nilsson G, Sundler F. Substance P-like immunoreactivity in intestinal carcinoid tumours. In: von Euler US, Pernow B, eds. *Substance P.* New York: Raven Press, 1977: 55–8.
5. Emson PC, Gilbert RFT, Mårtensson H, Nobin A. Elevated concentrations of substance P and 5-HT in plasma in patients with carcinoid tumours. *Cancer* 1983: in press.
6. Jaffe BM. Prostaglandins and serotonin: non-peptide diarrheogenic hormones. *World Journal of Surgery* 1979; **3:** 565–78.
7. Jager RM, Polk HC. Carcinoid apudomas. In: Hickey RC ed. *Current problems in cancer*, Vol. I. Chicago: Year Book Medical Publications Inc, 1977: 11.
8. Oates JA, Melmon K, Sjoersdsma A, Gillespie L, Mason DT. Release of kinin peptide in the carcinoid syndrome. *Lancet* 1964; **1:** 514–7.
9. Thorson A, Biörck G, Björkman G, Waldenström J. Malignant carcinoid of the small intestine with metastases to the liver, valvular disease of the right side of the heart (pulmonary stenosis and tricuspid requisation without septal defects) peripheral vasomotor symptoms, bronchoconstriction and an unusual type of cyanosis; a clinical and pathologic syndrome. *American Heart Journal* 1954; **47:** 795–817.
10. Waldenström J, Ljungberg E. Studies on the functional circulatory influence from metastasizing carcinoid (argentaffine, enterochromaffine) tumours and their possible relation to enteramine production. II. The chemistry of carcinoid tumours. *Acta Medica Scandinavica* 1955; **152:** 311–31.
11. Nobin A, Karp W, Lunderquist A, Rosengren E, Sandén G, Sundler F. Localization of carcinoids and pheocromocytomas with vein catheterisation and amine determination. *Brain Research Bulletin* 1982; **9:** 781–97.

12. Bengmark S, Ericsson M, Lunderquist A, Mårtensson H, Nobin A, Saco M. Temporary liver dearterialization of patients with metastatic carcinoids. *World Journal of Surgery* 1982; **6:** 46–53.
13. Mason RA, Steane PA. Carcinoid syndrome: its relevance to the anaesthetist. *Anaesthesia* 1976; **31:** 228–42.
14. Déry R. Theoretical and clinical considerations in anaesthesia for secreting carcinoid tumors. *Canadian Anaesthetists' Society Journal* 1971; **18:** 245–63.
15. Desmonts J-M, De Ribier A, Gilles M-R, Vic Dupont P. L'anesthésie et la réanimation dans la chirurgie des carcinoides digestifs secrétants. *Anesthesie, Analgesie, Reanimation* 1978; **35:** 143–53.
16. Stone HH, Donnelly CC. The anesthetic significance of serotonin secreting carcinoid tumors. *Anesthesiology* 1960; **21:** 203–12.
17. Jones WPG. Serotonin elease from carcinoid tumours. *Canadian Anaesthetists' Society Journal* 1962; **9:** 42–50.
18. Miller R, Patel AU, Warner RRP, Parnes IH. Anaesthesia for the carcinoid syndrome: a report of nine cases. *Canadian Anaesthetists' Society Journal* 1978; **25:** 240–4.
19. Kleine JW, Khouw YH, Heeres M. Management of anaesthesia during surgery for patients with carcinoid syndrome. *Acta Anaesthesiologica Belgica* 1977; **28:** 165–70.
20. Koehler LS, Christides PS, Adame TA. A complication of percutaneous cholangiography resulting in hypoxia and death of an anesthetized patient. *Anesthesiology* 1978; **49:** 210–2.
21. Patel KD, Dalal FY. Anesthetic management of a patient with carcinoid tumor undergoing myocardial revascularization. *Canadian Anaesthetists' Society Journal* 1980; **27:** 260–3.
22. Lippman M, Cleveland RJ. Anesthetic management of a carcinoid patient undergoing tricuspid valve replacement. *Anesthesia and Analgesia* 1973; **52:** 768–71.
23. Jones RM, Knight D. Severe hypertension and flushing in a patient with a non-metastatic carcinoid tumour. Hypertension and flushing with a solitary carcinoid tumour. *Anaesthesia* 1982; **37:** 57–9.
24. Carlsson C. Karcinoidsyndromets anestesiologiska betydelse. *Läkartidningen* 1969: 3657–66.
25. Olsson SG, Fletcher R, Jonson B, Nordström L, Prakash O. Clinical studies of gas exchange during ventilatory support—a method using the Siemens-Elema CO_2 analyzer. *British Journal of Anaesthesia* 1980; **52:** 491–9.
26. Neglén P. Aortic clamping. Skeletal muscle metabolism and central circulation during abdominal reconstructive vascular surgery for arteriosclerotic disease. *MD Thesis*, Lund, Sweden, 1980.
27. Jonson B, Nordström L, Olsson SG, Akerback D. Monitoring of ventilation and lung mechanics during automatic ventilation. A new device. *Bulletin de Physiopathologie Respiratoire* 1975; **11:** 729–43.
28. Kellermeyer RW, Graham RC. Kinins—Possible physiologic and pathologic roles in man. *New England Journal of Medicine* 1968; **279:** 754–9.
29. Grahame-Smith DG. The carcinoid syndrome. *American Journal of Cardiology* 1968; **21:** 376–87.
30. Hyatt M, Muldoon SM, Rorie DK. Droperidol, a selective antagonist of postsynaptic α-adrenoceptors in the canine saphenous vein. *Anesthesiology* 1980; **53:** 281–6.
31. Curran J, Crowley M, O'Sullivan G. Droperidol and endotracheal intubation. Attenuation of pressor response to laryngoscopy and intubation. *Anaesthesia* 1980; **35:** 290–4.
32. Toda N, Hatano Y. Contractile responses of canine tracheal muscle during exposure to fentanyl and morphine. *Anesthesiology* 1980; **53:** 93–100.
33. Burks TF, Long JP. Responses of isolated dog small intestine to analgesic agents. *Journal of Pharmacology and Experimental Therapeutics* 1967; **158:** 264–71.
34. Malavaud A, Pedinielli L, Quilichini R, Sethian M. Problems anaesthesiologiques posés par les carcinoides digestifs sécrétants a propos d'une observation. *Anesthesie, Analgesie, Reanimation* 1970; **27:** 657–66.
35. Baird WLM, Reid AM. The neuromuscular blocking properties of a new steroid compound, pancuronium bromide. A pilot study in man. *British Journal of Anaesthesia* 1967; **39:** 775–80.
36. Lee C, Yang E, Lippmann M. Constrictive effect of pancuronium on capacitance vessels. *British Journal of Anaesthesia* 1980; **52:** 261–3.
37. Andersen N. Changes in intragastric pressure following administration of suxamethonium. *British Journal of Anaesthesia* 1962; **34:** 363–7.
38. Pagé IH, McCubbin JW. The variable arterial pressure response to serotonin in laboratory animals and man. *Circulation Research* 1953; **1:** 354–62.
39. Robertson JIS, Peart WS, Andrews TM. The mechanism of facial flushers in the carcinoid syndrome. *Quarterly Journal of Medicine* 1962; **31:** 103–23.

HEART INVOLVEMENT IN METASTATIC CARCINOID DISEASE

Kenneth Törnebrandt, M.D.*; Jan Eskilsson, M.D., Ph.D.[O]; and
Anders Nobin, M.D., Ph.D.[X]

Departments of Anaesthesiology*, Cardiology[O] and Surgery[X],
University of Lund, Lund, Sweden

Running title: Carcinoid heart disease

Supported by the Swedish Medical Research Council (Grant No. 12X712)

Proofs and correspondance to: Kenneth Törnebrandt, M.D.
Department of Anaesthesiology
University Hospital
S-221 85 Lund
Sweden

SUMMARY

Sixteen patients with metastatic carcinoid tumors of ileal or caecal origin were studied in order to evaluate the frequency and degree of cardiac involvement in a nonselected patient group. We have also studied the correlation between plasma hormone levels (e.g., 5-hyroxytryptamine (5-HT) and substance P) and the degree of cardiac involvement. The patients underwent physical examinations, electrocardiograms, chest x-rays, cardiac catheterization, and echocardiography. Plasma levels of 5-HT and substance P were analyzed. Carcinoid heart involvement was found in 3 of 16 patients (19 %) but no patient had subjective symptoms associated with heart disease. Four patients (25 %) had a slight pulmonary hypertension. No left-sided heart lesions were seen. No correlation between blood levels of 5-HT or substance P and heart involvement was found. Eight patients died during the follow-up period, but in none of these was the cause of death cardiac failure. Carcinoid heart disease is not as common in our patients as in patients selected on a cardiological basis described in earlier studies. Echocardiography appears to be the most efficient technique for detection of even subclinical heart involvement and a useful tool for following its progress.

Keywords: Carcinoid heart, echocardiography, pulmonary stenosis, tricuspid insufficiency.

INTRODUCTION

The carcinoid syndrome occurs in patients with widespread metastatic carcinoid tumors. Extensive liver invasion by the tumor is invariably present. The symptoms involve several organ systems and are related to the production of pharmacologically active agents by the tumor itself (Sanders, 1973). The most prominent features of the syndrome include flushes, bronchiolar obstruction, lesions of the valves of the heart, and diarrhea (Mårtensson et al., 1983). When death is due to the carcinoid tumor, one of the main causes has been claimed to be cardiac failure, secondary to carcinoid heart disease (Thorson, 1958; Roberts and Sjoerdsma, 1964; Trell et al., 1973). There are few readily detectable signs of carcinoid heart disease. A precordial systolic murmur and a diffuse cardiac enlargement, visible on chest x-rays, may occur. An electrocardiogram (ECG) sometimes shows nonspecific changes, but the ECG is generally of little diagnostic help. Cardiac catheterization showing pressure changes compatible with tricuspid insufficiency and pulmonic stenosis has been used to diagnose carcinoid heart disease (Trell et al., 1973). Two-dimensional echocardiography is a recently developed promising technique for analyzing the degree of right-sided heart lesions (Howard et al., 1982).

In the present report we have performed echocardiography and heart catheterization in 16 consecutive patients treated in our respective departments for metastatic carcinoid disease of ileal or caecal origin. The aims of the study were to evaluate the frequency and degree of cardiac involvement in long-standing carcinoid disease and to correlate the plasma concentrations of hormones released from the tumor, such as 5-hydroxytryptamine (5-HT) and substance P, with pathological heart findings.

PATIENTS AND METHODS

Patients. The study group consisted of 16 consecutive patients with histologically proven carcinoid tumors of ileal or caecal origin and with liver metastases. They were referred to the Department of Surgery over a 4-year period. The patient group comprised 7 men and 9 women, aged 43-72 years (mean 62 years; Table I). Carcinoid syndrome with facial flushing and diarrhea was present in 12 patients. The mean duration of symptoms of the carcinoid disease prior to the heart investigation was 61 months (range 11-181 months). All patients had elevated levels of 5-HT in platelet-poor plasma (PPP) and whole blood as well as elevated excretion of 5-hydroxyindoleacetic acid (5-HIAA) in urine. Substance P in plasma was measured in 10 patients and raised levels were observed in 7 of them (Table I).

Clinical cardiac examination, chest x-ray and ECG were performed on all patients.

Echocardiography. Echocardiography was performed on 15 patients. An M-mode study was made in 4 cases (Organon Teknika Echocardiovisor 01). In 11 patients a two-dimensional (2-D) echocardiography study was obtained with a mechanical sector scanner (ATL, USA; 3-MHz transducer). Standard parasternal, apical and subcostal views were obtained (Popp et al., 1979) with the patient supine or in a left lateral decubitus position. Contrast echocardiography (Lieppe et al., 1978; Howard et al., 1982) was performed in 5 patients following injection of 10-15 ml of saline solution into a peripheral vein.

Doppler cardiography was used in 3 cases to evaluate possible tricuspid insufficiency. The tricuspid valve area and the right atrium were searched for systolic regurgitant flow with a pulsed Doppler unit (ATL, USA) (Miyatake et al., 1982).

Heart catheterization. All patients were subjected to surgery or liver-embolization aimed at reducing the carcinoid tumor volume. During these procedures, 14 of the patients were invasively monitored. An arterial and a triple lumen thermistor pulmonary catheter (Swan-Ganz$^{(R)}$) were inserted under local anaesthesia. During catheter withdrawal pressure recordings were obtained from the pulmonary artery, the right ventricle, and atrium.

Table I. Patient data

Patient	Age (yr) and sex	Site of primary lesion	Carcinoid syndrome	5-HT in PPP (ng/ml)	5-HT in whole blood (ng/ml)	5-HIAA in urine (μmol/24 hr)	Substance P in plasma (pg/l)	Duration (mo) of symptoms prior to investigation	Observation time (mo) after investigation	Autopsy
1	72 M	ileum	+	199	608	165	-	36	21 †	-
2	64 F	caecum	-	140	949	330	-	181	13 †	-
3	68 F	ileum	-	48	1252	2075	88	26	16 †	Thickened tricuspid leaflets with fibrous plaques
4	60 F	ileum	+	78	499	420	-	16	11 †	-
5	63 F	ileum	+	42	425	90	10	16	39 †	Slightly atheromatotic valves
6	43 M	ileum	+	57	2041	2030	283	96	7 †	-
7	60 F	ileum	+	187	3466	2695	68	60	16	
8	56 M	ileum	-	18	390	80	26	75	16	
9	62 M	ileum	+	124	808	475	11	70	1 †	-
10	72 F	ileum	+	1251	1599	2140	43	83	10	
11	62 M	ileum	-	412	1504	972	-	15	4	
12	64 M	ileum	+	26	561	341	72	27	4	
13	64 F	ileum	+	12	487	102	-	73	1	
14	62 F	ileum	+	21	748	263	7	70	22	
15	55 M	ileum	+	32	1220	615	20	63	16	
16	61 F	caecum	+	18	1190	1548	-	120	14 †	-
				normal < 9	normal < 250	normal < 80	normal < 20			

Biochemistry. 5-HT determinations in PPP and whole blood were performed with a high performance liquid chromatography (HPLC) technique with electrochemical detection (Nobin et al., 1982). In urine, 5-HIAA was measured by a spectrophotometric method (MacFarlane et al., 1956). Substance P in plasma was determined by radioimmunoassay (Nobin et al., 1983).

The study was performed with the informed consent of the patients and was approved by the Ethical Committee of the University of Lund.

RESULTS

Clinical Findings

At the time of the examination, 4 patients experienced a slight malaise, but otherwise the patients' general condition was good. One patient suffered from shortness of breath but no other patient complained of symptoms which objectively could be associated with heart disease.

The results of the clinical examination, ECG recordings, and chest x-rays are summarized in Table II. A pathologic murmur was found in one patient (No. 14). The ECG was normal in 9 patients. Three patients (Nos. 5, 12 and 14) had nonspecific ST-T depressions over the anterior wall. One patient had atrial fibrillation and right bundle-branch block (No. 1), and in another (No. 4) paroxysmal atrial fibrillation was found. A left anterior hemiblock was seen in two patients (Nos. 10 and 13).

Chest x-rays were normal in 12 patients. In the remaining 4 patients (Nos 1, 7, 11 and 15) a slight to moderate left ventricular enlargement was found. Signs of pulmonary congestion were not observed in any patient.

Invasive Blood Pressure Measurements

The results of the pressure recordings performed during right heart catheterization are presented in Table II. A slight pulmonary hypertension was found in 4 patients (Nos. 6, 8, 11 and 12). In one patient (No. 2) a pressure gradient of 21 mm Hg over the pulmonary valve was registered, consistent with a mild pulmonary stenosis. There was no end-diastolic pressure gradient over the tricuspid valves in any patient. No giant "v"-

wave were seen in the right atrial tracings and the "v"-wave did not exceed the "a"-wave in any case. The morphology of the pressure curves was normal in all patients, and the curves showed a distinct "x"-descent.

Echocardiographic Findings

With M-mode echocardiography, signs of right ventricular overload (RVO) were seen in 2 patients (Nos. 2 and 14, Table III). In Patient No. 14, 2-D echo revealed thickened and immobile tricuspid valve leaflets, while normal tricuspid leaflets were found in the remaining 10 patients examined with 2-D echo. The pulmonary cusps, visualized with 2-D echo in 7 patients, showed a normal echocardiographic appearance. With contrast echocardiography and/or Doppler cardiography, signs of tricuspid regurgitation were found in one of 7 examined patients (No. 14). The left-sided valves were normal in all 15 patients examined with echocardiography.

Clinical Course

Eight patients died during the follow-up period (Table I). The mean time from the heart investigation to death was 15 months (range 1-39 months). Autopsy was performed in 2 patients, one of whom had fibrous plaques on thickened tricuspid valve leaflets consistent with carcinoid heart disease (No. 3). The other patient had slightly atheromatotic valve lesions without correlation with carcinoid disease. Although autopsy was not performed on the remaining 6 patients, the available clinical data indicated that the probable cause of death was the carcinoid malignant growth rather than cardiac failure. The other 8 patients are still alive, with a mean observation time of 11 months (range 1-22 months) after the investigation.

Table II. Results of clinical examination and invasive measurements.

Patient	Murmur	ECG	Chest X-ray	Pressures (mm Hg)							
				Radial artery		Pulmonary artery		Right ventricle		Right atrium	
				s	d	s	d	s	id/ed	a	v
1	0	AF,RBBB	Moderate LVE	101	63	17	10	18	2/5	5	4
2	0	N	N	170	115	15	8	36	8/10	12	10
3	-	N	N	97	55	21	8	22	4/6	6	5
4	Faint syst apex	PAF	N	140	85	22	8	19	3/5	4	4
5	Faint syst apex	NSST-T	N	111	82	26	14	27	7/12	12	10
6	0	N	N	147	88	37	14	36	4/7	7	7
7		N	Moderate LVE	142	78	17	-1	21	neg	2	0
8	0	N	N	135	70	34	14	33	5/10	10	8
9	0	N	N	175	95	18	6	20	0/3	4	3
10	Faint syst left parasternal border	LAH	N	158	69	29	11	31	neg/7	9	6
11	0	N	Slight LVE	177	103	37	18	40	5/11	11	10
12	0	NSST-T	N	195	100	38	18	34	0/5	4	3
13	0	LAH	N	195	100	21	11	22	3/5	8	6
14	Moderate syst High-pitched diast left parasternal border	NSST-T	N	-	-	-	-	-	-	-	-
15	0	N	Slight LVE	165	85	29	13	33	3/9	9	6
16	0	N	N	-	-	-	-	-	-	-	-

Abbreviations: AF = atrial fibrillation; RBBB = right bundle-branch block; N = normal; PAF = paroxysmal atrial fibrillation; NSST-T = non specific ST-T depression; LAH = left anterior hemiblock; LVE = left ventricular enlargement; s=systolic; d=diastolic; id=initial diastolic; ed=end-diastolic; a=a-wave; v=v-wave.

Table III. Echocardiographic data.

Patient	Echocardiography: M-mode RVO	Echocardiography: 2-D echo: Tricuspid leaflets	Echocardiography: 2-D echo: Pulmonic cusps	Echocardiography: 2-D echo: Contrast echo (TR)	Doppler cardiography (TR)
1	No	-	-	-	-
2	Yes	-	-	-	-
3	-	-	-	-	-
4	No	-	-	-	-
5	No	-	-	-	-
6	No	N	Not visualized	Negative	-
7	No	N	N	Negative	-
8	No	N	N	-	-
9	No	N	N	-	Negative
10	No	N	N	Negative	-
11	No	N	N	-	-
12	No	N	N	-	-
13	No	N	N	-	Negative
14	Yes	Thickened, immobile	Not visualized	Positive	-
15	No	N	Not visualized	Negative	Negative
16	No	N	Not visualized	—	—

Abbreviations: RVO = right ventricular overload; N = normal; TR = tricuspid regurgitation.

DISCUSSION

Carcinoids of the gut arising in areas other than the appendix should be considered true cancerous lesions with lethal potential (Mårtensson et al., 1983). When liver metastases are present the median survival time is about 24 months (Moertel et al., 1961). The malignant carcinoids disable the patients both with the tumor burden as such, with general cachexia, and with the widespread effects of the hormones released from the tumor. The excess of hormones such as 5-HT, substance P, and bradykinins released into the circulation (Nobin et al., 1982; 1983; Mårtensson et al., 1985) cause premature death from cardiac and nutritional failure. In fact, cardiac complications have been claimed to be one of the most important lethal factors in carcinoid syndrome (Thorson et al., 1958; Robertson and Sjoerdsma, 1964; Trell et al., 1973; Costello, 1975). In a study of 56 carcinoid patients Thorson and collaborators demonstrated that heart failure was the major cause of death in 16 cases and a contributory cause in another 27 patients (Thorson et al., 1958). Contradictory findings were recently published by Howard and collaborators (Howard et al., 1982). They investigated and followed 14 patients with carcinoid syndrome and, although 8 patients had definite abnormalities of the right-sided cardiac valves, no patient died as a direct result of carcinoid heart disease.

Heart disease thus appears to be an important cause of death in patients with malignant endocrine tumors of the gut. The discrepancy in frequency of heart involvement in different reports can be explained by the different methods used for selecting patients. In some investigations subjective cardiac symptoms or heart murmurs have been the criteria for including patients in the study, whereas in other investigations all patients with malignant carcinoids have been included.

Our study comprised 16 consecutive patients with malignant carcinoids of midgut origin referred to us for surgical treatment of the carcinoid tumor. All patients had widespread disease with elevated plasma hormone levels. Twelve patients suffered from the carcinoid syndrome with flushes and diarrhea, but only one patient complained of cardiac symptoms. Chest radiographs and ECGs revealed no unequivocal cardiac abnormality in our patients, and the limited value of these investigations in suspected carcinoid heart disease has been pointed out previously (Howard et al., 1982). The ECGs may show low voltage or signs of right atrium and/or

ventricular enlargement, but the findings are usually inconsistent and non-specific (compare Patients 2 and 14 in our study) (Roberts and Sjoerdsma, 1964; Trell et al., 1973).

To evaluate the frequency of subclinical heart involvement in patients with malignant carcinoids we have chosen to use 2-D echo and heart catheterization with pressure measurements.

Cardiac catheterization with direct measurement of pressures is usually reliable in the diagnosis of pulmonary valve stenosis, but mild tricuspid valve lesions are difficult to rule out with a single catheter (Mendel, 1968). Morphological changes of the tricuspid leaflets are best diagnosed by 2-D echo, and contrast echocardiography or Doppler cardiography are probably the methods of choice for the early detection of tricuspid regurgitaion (Lieppe et al., 1978; Miyatake et al., 1982).

In our study, involvement of the right-sided heart valves was seen in 3 patients. In one of these (No. 3) the carcinoid heart involvement was found at the autopsy. The negative invasive pressure measurements obtained 16 months prior to death may be due either to the absence of lesions or to their lack of hemodynamic significance at that time. In Patient No. 2, who had an invasively proven mild pulmonic stenosis, the M-mode echo was also abnormal showing RVO. In Patient No. 14, the echocardiographic findings were typical for carcinoid atrioventricular involvement, with thickened tricuspid leaflets frozen in a semiopen position (Callahan et al., 1982).

The low incidence of carcinoid heart involvement found in the current study (3/16 = 19 %) appears to be in contradiction to the findings in recent reports by Callahan et al. (1982), Howard et al. (1982) and Forman et al. (1984) in which right-sided valve lesions were observed in 43-57% of the patients. In their series, however, all patients without clinical signs of valve lesions were found to be normal by echocardography. Thus, patient selection should be considered when the incidence of heart involvement is compared.

The relative ratio between pulmonic stenosis and tricuspid regurgitation in our study was similar to that reported by other workers (Roberts and Sjoerdsma, 1964; Trell et al., 1973). A predominance of pulmonic stenosis has, however, been found in some earlier reports (e.g., Thorson et al.,

1954). In these studies, the type of heart involvement was based on autopsy findings. With modern noninvasive diagnostics it may be easier to detect tricuspid regurgitation than pulmonic stenosis, raising the question of whether pulmonic involvement has been underestimated in some recent studies. Our results, however, do not support the occurrence of clinically silent pulmonary stenosis.

Pulmonary hypertension has rarely been described in carcinoid patients with high blood 5-HT concentration (Charms et al., 1959), which is surprising considering the striking effect of 5-HT on pulmonary artery pressure that has been found in animal experiments (Rudolph and Paul, 1957; Wanner et al., 1978). A possible explanation could be that right-sided cardiac lesions can have a protective effect against raised pulmonary artery pressure (Charms et al., 1959). In the present study we found mild pulmonary hypertension in 4 patients (4/14 = 29 %). These data must be interpreted with caution, however, since in 3 patients the pressure recordings were obtained at the end of hepatic embolization under local anaesthesia. The ischemia resulting from hepatic embolization causes pain, which may induce a hemodynamic strain, and the raised systolic pulmonary artery pressures recorded in our patients (34-38 mm Hg) did not exceed the upper normal limit for patients undergoing a work test. In a study of 16 patients who also had liver metastases and were undergoing surgery, no case of pulmonary hypertension was registered (Törnebrandt et al., 1983).

Left-sided heart lesions are rarely seen in carcinoid heart disease (Trell et al., 1973; Strickman et al., 1982), although in one report as many as 3 of 9 patients had mitral or aortic involvement (Roberts and Sjoerdsma, 1964). In our study no left-sided heart lesions were found. This is as expected because, in cases where no right to left shunt exists, the left heart is only exposed to a "spillover" of vasoactive substances after the lung passage.

Although the heart lesions have been attributed to high levels of 5-HT (Thorson et al., 1954; Roberts and Sjoerdsma, 1964; Trell et al.,1973), we did not find any correlation between the measured high levels of blood 5-HT or urinary 5-HIAA levels and the degree of heart involvement. Neither have we been able to correlate the duration of the disease with heart lesions, which is in accordance with the findings of Howard et al. (1982). The pathophysiology of the cardiac lesions is thus probably dependent upon

different sensitivity between the patients as well as interaction between the different substances released from the endocrine tumors.

In conclusion, we have found a relatively low incidence of heart involvement in nonselected patients with metastasizing midgut carcinoid tumors. None of our patients have died of cardiac failure. Against the background of the important clinical implications of heart involvement, however, we recommend a careful clinical and echocardiographic examination of the heart in all carcinoid patients.

REFERENCES

Callahan JA, Wroblewski EM, Reeder GS, Edwards WD, Seward JB, Tajik AJ: Echocardiographic features of carcinoid heart disease. Am J Cardiol 50, 762 (1982).

Charms BL, Kohn P, Applebaum HI, Geller J: Hemodynamic studies in a case of carcinoid cardiovascular syndrome. Circulation 20, 208 (1959).

Costello C: Carcinoid tumor metastases - prospective study of twenty-two patients. Am J Surg 130, 756 (1975).

Forman MB, Byrd BF, Oates JA, Robertson RM: Two-dimensional echocardiogaphy in the diagnosis of carcinoid heart disease. Am Heart J 107, 492 (1984).

Howard RJ, Drobac M, Rider WD, Keane TJ, Finlayson J, Silver MD, Wigh ED, Rakowski H: Carcinoid heart disease: Diagnosis by two-dimensional echocardiography. Circulation 66, 1059 (1982).

Lieppe W, Behar VS, Scallion R, Kisslo JA: Detection of tricuspid regurgitation with two-dimensional echocardiography and periferal vein injections. Circulation 57, 128 (1978).

MacFarlane PS, Dalgliesh CE, Dutton RW, Lennox B, Nyhus LM, Smith AN: Endocrine aspects of argentaffinoma with special reference to the use of urinary 5-hydroxyindoleacetic acide estimations in diagnosis. Scott Med J 1, 148 (1956).

Mendel D (Ed.): A practice of cardiac catheterization. Blackwell Scientific publications (1968), p.234.

Miyatake K, Okamoto M, Kinoshita N, Ohta M, Kozuka T, Sakakibara H, Nimura Y: Evaluation of tricuspid regurgitation by pulsed doppler and two-dimensional echocardiography. Circulation 66, 777 (1982).

Moertel CG, Sauer WG, Dockerty MB, Bagenstoss AH: Life history of the carcinoid tumor of the small intestine. Cancer 14, 901 (1961).

Mårtensson H, Nobin A, Sundler F: Carcinoid tumors in the gastrointestinal tract - an analysis of 156 cases. Acta Chir Scand 149, 607 (1983).

Mårtensson H, Nobin A, Sundler F, Falckmer S: Endocrine tumors of the ileum - cytochemical and clinical aspects. Pathol Res Pract. In press (1985).

Nobin A, Karp W, Lunderquist A, Rosengren E, Sandén G, Sundler F: Localization of carcinoids and pheochromocytomas with vein catheterization and amine determination. Brain Res Bull 9, 781 (1982).

Nobin A, Emson P, Mårtensson H, Sundler F: Substance P in carcinoid patients - tumour content and plasma levels. In Substance P Eds. Skrabanek P and Powell D. Boole Press, Dublin (1983), 129.

Popp RL, Fowles RE, Coltart DJ, Martin RP: Cardiac anatomy viewed systematically with two-dimensional echocardiography. Chest 75, 579 (1979).

Roberts WC, Sjoerdsma A: The cardiac disease associated with the carcinoid syndrome (carcinoid heart disease). Am J Med 36, 5 (1964).

Rudolf AM, Paul MH: Pulmonary and systemic vascular response to continuous infusion of 5-hydroxytryptamine (serotonin) in the dog. Am J Physiol 189, 263 (1957).

Sanders RJ: Carcinoids of the gastrointestinal tract. Charles C Thomas - publisher. Springfield, Illinois, USA. 3 (1973).

Strickman NE, Rossi PA, Massumkhani GA, Hall RJ: Carcinoid heart disease: A clinical pathologic and therapeutic update. Curr Probl Cardiol 6, 11 (1982).

Thorson Å, Björck G, Björkman G, Waldenström J: Malignant carcinoid of the small intestine with metastases to the liver, valvular disease of the right side of the heart (pulmonary stenosis and tricuspid regurgitation without septal defects), peripheral vasomotor symptoms, bronchoconstriction, and an unusual type of cyanosis. Am Heart J 47, 795 (1954).

Thorson Å: Studies on carcinoid disease. Acta Med Scand suppl 334 (1958).

Trell E, Rausing A, Ripa J, Torp A, Waldenström J: Carcinoid heart disease. Clinicopathologic findings and follow-up in 11 cases. Am J Med 54, 433 (1973).

Törnebrandt K, Nobin A, Ericsson M, Thomson D: Circulation, respiration and serotonin levels in carcinoid patients during neurolept anaesthesia. Anaesthesia 38, 957 (1983).

Wanner A, Begin R, Cohn M, Sackner MA: Vascular volumes of the pulmonary circulation in intact dogs. J Appl Physiol 44, 956 (1978).

Pharmacological Characterization of Alpha-Adrenergic Receptor Subtypes Mediating Contraction in Human Mesenteric Arteries and Veins

Kenneth Törnebrandt, Anders Nobin and Christer Owman

Departments of Anesthesiology, Surgery, and Histology, University of Lund, S-221 85 LUND, Sweden

Running title: α-adrenergic receptors in mesenteric vessels

Acknowledgements of support: The study was supported by grants from the Swedish Medical Research Council (grants No 14X-732 and 12X-712), the Magnus Bergwall Foundation and the Albert Påhlsson Foundation

Proofs and correspondence to: Kenneth Törnebrandt, M.D., Department of Anesthesiology, University of Lund, S-221 85 LUND, Sweden

Abstract

Approximately 200 isolated human mesenteric arteries (diameter less than 1 mm) and veins (diameter less than 2 mm) were tested in vitro for their contractile and dilator responses to adrenergic agonists under standardized conditions. This allowed for quantitative estimation of various receptor characteristics: relative agonist potency, concentration for half maximum response (EC_{50}), amount of maximum response (E_{Am}), and affinity of antagonist to receptor (K_B and pA_2). The contractile response of the vessels to the various sympathomimetic amines involved α-adrenoceptors. Postjunctionally, the α_1-type of receptor predominated in the arteries whereas the α_2-receptor subtyp was mainly found in the veins. None of the amines produced any vasodilatory effects in the vessels tested after previous contraction with prostaglandin $F_2\alpha$.

Key Words: Human mesenteric arteries and veins; Smooth muscle contraction in vitro; Quantitative pharmacology; Sympathomimetic amines; Adrenoceptor antagonists; Adrenoceptors.

Introduction

The gastrointestinal tract represents a large tissue mass with complex function, including smooth muscle motility, glandular secretion, and absorption from a huge surface of epithelial cells. The splanchnic vascular bed therefore receives as much as 25% of cardiac output, which corresponds to nearly 1,500 ml/min in a 70-kg man. It plays a central role in clinical medicine and has provided an extensively used model in circulation research (Folkow and Neil, 1971). The various functional components are highly sensitive to circulatory disturbances under a wide range of conditions, from hypovolemic shock (Lillehei and Washington, 1957) to the release of high concentrations of vasoactive transmittors from abdominal endocrine tumors (Törnebrandt et al., 1983).

In spite of a great interest in the mechanisms by which the human splanchnic circulation is influenced by vasoactive amines and peptides - released locally from nerves and endocrine tissue or circulating in the blood - only a few studies have been concerned with basic functional and pathophysiological circulatory problems in this vascular bed of man (Samnegård et al., 1980; Thulesius, 1983). The present study on isolated human mesenteric arteries and veins in vitro was undertaken to characterize quantitatively the adrenergic receptors mediating vasoconstriction and dilatation. The aim has particularly been to increase our understanding about the vascular reactions that may occur in the splanchnic circulation in patients with elevated blood catecholamine levels due to severe stress or trauma, or originating from endocrine tumors, such as pheochromocytomas. This will provide a model for a better interpretation of circulatory disturbances that may take place also in other vascular beds.

Material and Methods

Small segments of mesenteric arteries and veins were obtained from a total of approximately 100 men and women undergoing surgery due to non-endocrine ventricular or intestinal malignancy. The patients were otherwise healthy and their age ranged between 20 and 70 years. Morphine chloride and scopolamine bromide, or droperidol and fentanyl, were given as preanesthetic medication. Anesthesia was induced by pentothal sodium, or droperidol together with fentanyl administered intravenously. Intubation was facilitated by succinylcholine, and the patients were ventilated with dinitrous oxide and oxygen. Supplementary doses of pancuronium bromide and fentanyl were given as needed. The patients were informed in detail about the purpose of the investigation and had given their consent to the excision of the vascular preparations.

Vascular Preparations

The vessels were dissected from terminal branches of the mesenteric vessels close to the intestines. Attempts were made to remove arteries with a diameter of 1 mm or less (0.4-0.9 mm) and veins 2 mm or less (0.5-1.7 mm). The vessels were removed with an atraumatic technique and the ischemic period was less than 20 s. They were then immediately transferred to a cold, modified Krebs-Ringer solution (for composition, see below) and kept at +4 ^{o}C in a refrigerator until the experiments were performed on the same day or the subsequent 2 days. A 5-mm long piece of the vessel was prepared free from surrounding connective tissue and mounted between two L-formed metal holders in a 50-ml manteled organ bath for recording of circular motor activity (Edvinsson et al., 1974). Small pieces of the vessels were fixed in Bouin´s solution (formalin:picric acid:glacial acetic acid, 5:15:1). Transverse paraffin sections of 5 μm thickness were stained in hematoxyline and eosin (Baker, 1970). Light microscopic examination was used for measuring wall thickness allowing estimation of the passive load to be given to the vessels in vitro. On the basis of Laplace´s law, wall tension is equal to the product of the intraluminal pressure and the radius divided by the wall thickness (Frank, 1920).

The organ bath contained a modified Krebs-Ringer solution of the following composition (mM concentration): NaCl 118; KCl 4.5; $CaCl_2$ x 2 H_2O 2.5;

$MgSO_4$ x 7 H_2O 1.0; $NaHCO_3$ 25; KH_2PO_4 1.0, and glucose, 6.0. The temperature in the bath was thermostatically maintained at +37 °C and the buffer solution was continuously aerated with a mixture of 95% O_2 and 5% CO_2 giving pH 7.40 (range 7.35-7.45). The bath contained 10^{-6} M each of cocaine and normetanephrine to block neuronal and extraneuronal uptake, respectively, of the amines (Iversen, 1967). The contractile and dilator responses were recorded isometrically with Grass FT03C force-displacement transducers and monitored on a Grass 79D polygraph.

In vitro Recordings

In one series of experiments the influence of different degrees of initial passive tension applied to mesenteric arteries or veins were studied. After mounting, the vessels were given an initial passive tension of 5-20 mN (corresponding to a weight calibration of 0.5-2.0 g) after which they were allowed to accomodate for 60-90 min. The tension was then increased stepwise up to 80-90 mN in a cumulative way. At each tension the effect of 10^{-6} M (i.e. close to EC_{50} in order to avoid vascular damage) norepinephrine was tested, in the presence of 10^{-6} M propranolol to block any dilatory β-adrenergic receptors. The contraction was recorded, the amine was washed out and the vessel allowed to relax to each level of original tension.

In a second series of experiments, changes in the sensitivity to norepinephrine were studied in the same vessel used either 1-2 h after operation or after storage for 1 or 2 days in a buffer solution at +4 °C. The sensitivity to norepinephrine was also tested over a period of several hours.

In a third series of experiments the contractile and dilatory responses were tested to various agonists and antagonists. Agonists were added to the bath in a cumulative manner (van Rossum and van den Brink, 1963) in order to obtain full concentration-response curves since pilot experiments had shown that fractionated application gave identical results (fig. 1). When contractile activity was tested, 10^{-6} M propanolol was present in the bath in order to block any β-receptor mediated dilatory effects. When dilatory responses were studied the vessels were given an active tone by 10^{-6} to 10^{-5} M prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and

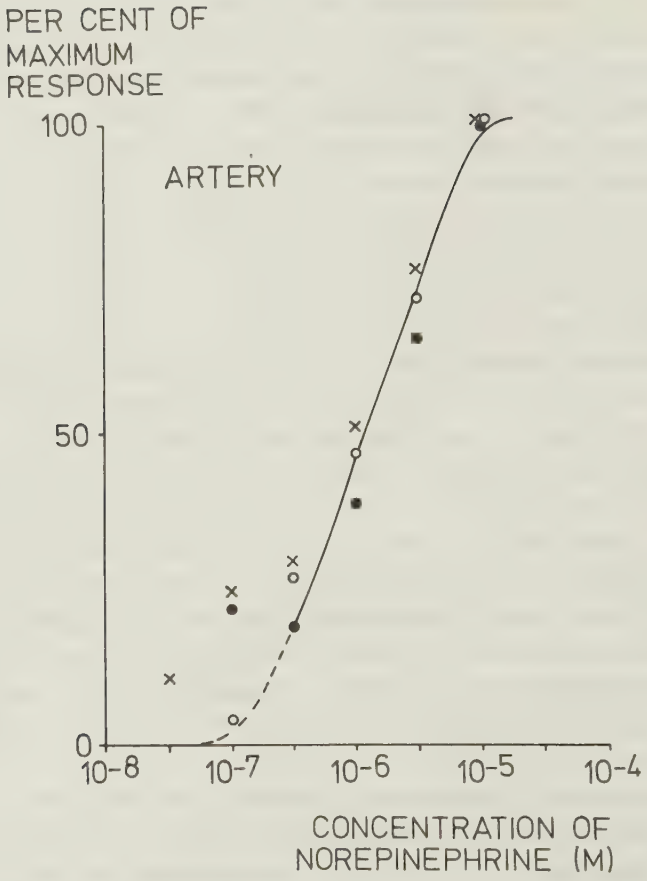


Fig. 1 Representative example of identical contractile response in two mesenteric arteries following administration of norepinephrine by either cumulative application (•) or fractionated application (o). In the latter case the agonist was washed out between each single, increasing concentration.

the contractile α-receptors were blocked with 10^{-6} M phenoxybenzamine. In some cases, potassium-rich buffer solution (final K^+ concentration 5×10^{-2} M) was used to produce vasoconstriction. The contraction achieved in either way was found to remain at a steady level for at least 30 min.

Drugs

The following compounds were tested: L-arterenol hydrochloride, epinephrine bitartrate, L-isoproterenol hydrochloride; L-phenylephrine hydrochloride (Sigma); clonidine hydrochloride (Boehringer); oxymetazoline chloride (Draco); methoxamine hydrochloride (Burroughs, Wellcome); carbamylcholine chloride (Aldrich); $PGF_{2\alpha}$ (Amoglandin, Recip); phentolamine (Regitin, Ciba-Geigy); piperoxan hydrochloride (Rhône-Poulenc); yohimbine hydrochloride (Sigma); prazosin hydrochloride (Pfizer); phenoxybenzamine hydrochloride (Sigma), terbutaline sulfate (Bricanyl, Draco); propranolol hydrochloride (Inderal, ICI); cocaine hydrochloride (ACO); normetanephrine hydrochloride, and substance P (Sigma). $PGF_{2\alpha}$ was dissolved in 1% benzyl alcohol, prazosin hydrochloride in 10% lactic acid. All other substances were dissolved in 0.9% saline. The catecholamine solutions contained 0.2 mg/ml ascorbic acid to minimize oxidation. The concentrations below are given as final molar concentration in the bath.

Analysis of Data

The pharmacological tests were used to estimate maximum response (E_{Am}), concentration of agonists giving half maximum response (EC_{50}), relative agonist potency, and the affinity of certain antagonists for corresponding receptors (K_B and pA_2). Curves were plotted in terms of relative vasomotor response also in the blocking experiments, since none of the antagonists caused a decrease in maximum or slope of the actual concentration-response curves. According to the occupation theory of drug-receptor interaction (Furchgott, 1972), the formation of the complex between the receptor and the agonist is governed by the law of mass-action. The response of a test preparation to an agonist under steady-state conditions is considered to be a function of the concentration of the receptor-agonist complex times an efficacy term for this specific complex (Stephenson, 1956). Besides determining the relative potency by which various agonists produced a response, and the inhibition of this response by low doses of specific antagonists, the receptor was further characterized by estimation of the dissociation constant for the receptor-antagonist complex (K_B). This expresses the relation between the concentration of antagonist and dose ratio minus 1 (dr - 1). The calculation requires that the values of (dr - 1) for the agonist against the concentration of the antagonist, plotted in their

logarithmic forms, fit a straight line with a slope of unity (Arunlakshana and Schild, 1959). The intercept of this line with the abscissa represents the pA_2 value, which is the logarithm for the concentration of the antagonist required to increase the EC_{50} value by a factor of 2 (Schild, 1947).

Student's t-test for unpaired and paired observations was used to compare mean values. Regression lines were obtained by the method of least squares.

Results

General mechanical properties

The influence on the contractile response of mesenteric arteries caused by variations in the initial tension of the vessel was tested. The amount of contraction induced by 10^{-6} M norepinephrine is illustrated in figure 2a. An initial load in the range of 15-40 mN gave a maximum contraction. For the further pharmacological experiments an initial tension ranging between 20 and 40 mN, depending on gross estimations of arterial diameter,

Table I. Various quantitative parameters of mesenteric arteric arteries and veins related to dimension and estimated blood pressure in situ (mean ± SEM)

Parameters	Mesenteric arteries	Mesenteric veins
Vessels diameter, μm	640 ± 70 (n = 8)	810 ± 140 (n = 8)
Wall thickness, μm	130 ± 25 (n = 8)	110 ± 16 (n = 8)
Estimated local blood pressure, mm Hg	90	15
Estimated transmural pressure, mN/mm^2	12	2
Estimated tangential pressure, nM/mm^2	25	8
Passive load in organ bath, mN	35 ± 8 (n= 8)	27 ± 4 (n = 8)
Estimated vessel tension in organ bath, mN/mm^2	4	2

was therefore chosen. For mesenteric veins an initial load of 20-35 mN gave maximal contractile response (fig. 2b), and approximately 25 mN was chosen for the experiments. The levels of passive load given to the vessels in vitro were well below the amount of tension the arteries and veins are exposed to in vivo (table I) by which overstretching was avoided.

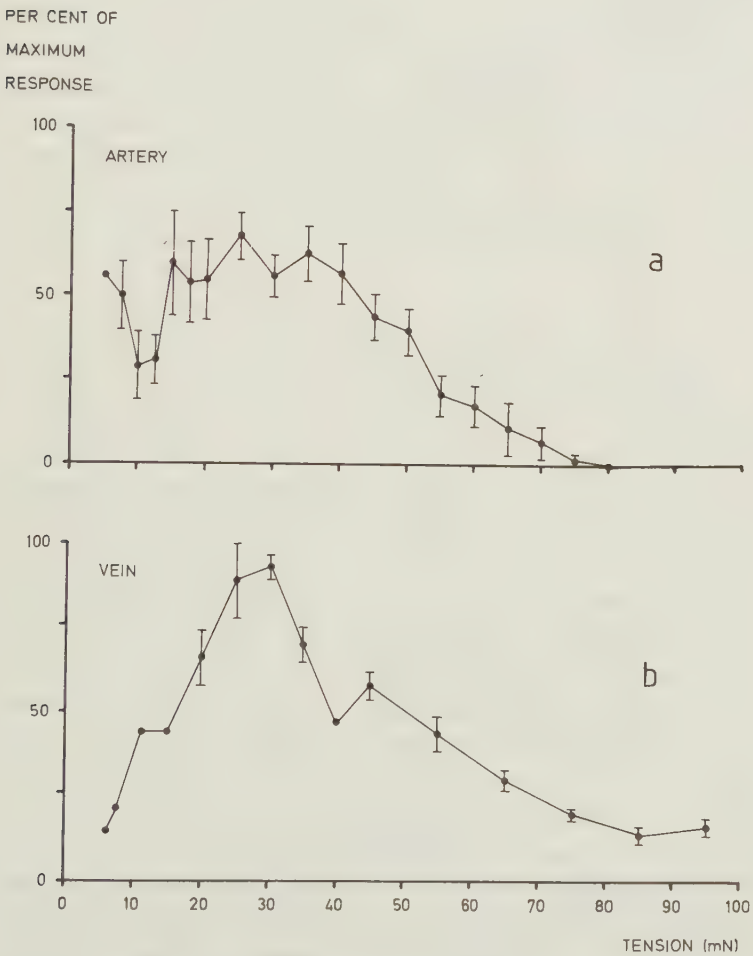


Fig. 2 Influence of different degrees of initial passive tension on the contractile response to 10^{-6} M norepinephrine tested in 5 mesenteric arteries (a) and 4 mesenteric veins (b). Values are means ± SEM.

Control studies over the test period showed that the EC_{50} values and E_{Am} for a given agonist remained constant with time. Storage for 24 or 48 h in buffer solution at +4 °C did not interfere with the smooth muscle reactivity as tested with norepinephrine (fig. 3).

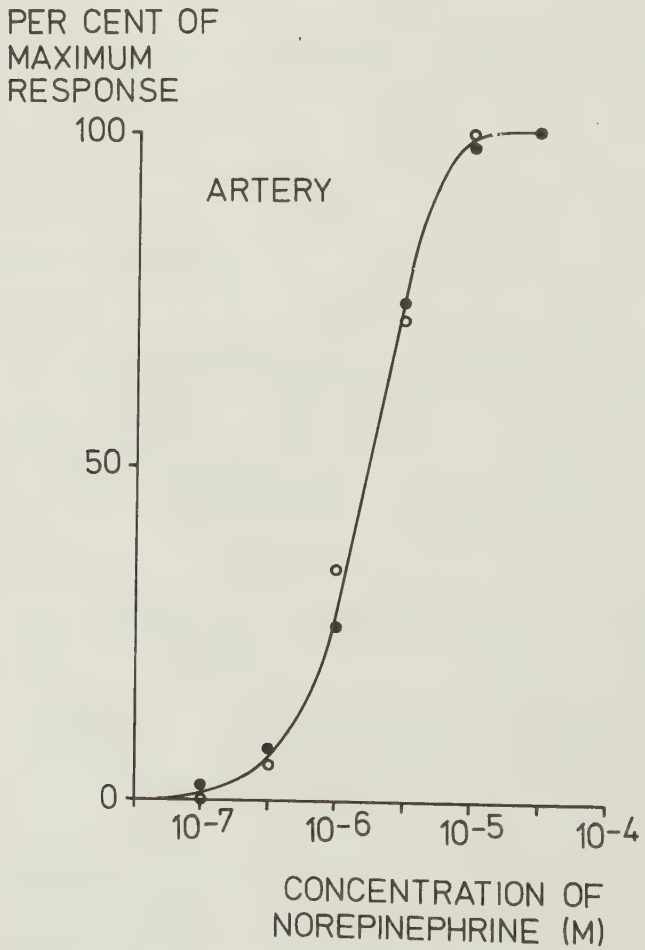


Fig. 3 Norepinephrine-induced response in 3 different pieces of mesenteric artery from the same patient following various storage times in buffer solution at +4 °C. The preparations were tested after storage for 1 h (●), 24 h (o) or 48 h (x). The effect at the lowest concentrations sometimes did not follow the sigmoid curve, otherwise the response is near identical following the three storage periods.

The relative potency based on the EC_{50} values (table II) for the sympathomimetic contractions in the arteries was in the order oxymetazoline > epinephrine > norepinephrine > phenylephrine > methoxamine > isoproterenol. These differences were all statistically significant ($p < 0.001$). This suggests that the response is mediated by α-adrenergic receptors (Furchgott, 1972) which was confirmed by the shift of the concentration-response curve for norepinephrine towards higher concentrations, without appreciable effects on E_{Am}, in the presence of increasing concentrations of phentolamine (fig. 4a). The parallel nature of the shift was confirmed in the Schild plots giving a straight line with a slope not significantly different from unity ($p > 0.05$; fig. 4b). The intercept of the regression line with the abscissa (giving the pA_2 value) was 8.5 (fig. 4b). The corresponding mean K_B value for phentolamine was $(1.9 \pm 0.9) \times 10^{-8}$ M (table II).

Attempts were made to classify the postjunctional α-receptor in terms of α_1 and α_2 subtypes. The α_2-selective antagonists, yohimbine (10^{-8}, 3×10^{-8} or 10^{-7} M) and piperoxan (at concentrations up to 10^{-6} M), had no effect on the contractile response produced by norepinephrine. On the other hand the α_1-antagonist, prazosin, caused a parallel shift showing true competitive antagonism as confirmed in Schild plots (fig.5). In accordance with these inhibition experiments, methoxamine contracted the mesenteric arteries, though the maximum showed considerable variation, whereas the α_2-receptor agonist, clonidine, was entirely without contractile effect in 14 of 18 vessels tested in concentrations ranging between 10^{-9} and 10^{-3} M in the bath (fig. 6). However, oxymetazoline, which is classified as an α_2-selective agonist, regularly contracted the arteries, though the range of E_{Am} was considerably wider than with the other agonists tested (except methoxamine). Oxymetazoline showed the lowest EC_{50} value among the agonists with contractile effects. The contractile action of 3×10^{-7}M oxymetazoline (approximatively EC_{50}) was inhibited by 10^{-7} M prazosin, but not by the same dose of yohimbine (fig. 6). The results of the various quantitative agonist responses and figures for the interaction with different antagonist are summarized in table II.

Table II. Pharmacological in vitro analysis of quantitative characteristics of adrenergic contractile mechanisms in human mesenteric arteries responding to a particular agonist (mean ± SEM).

Amine tested	EC_{50}, M	Relative potency	E_{Am}, mN	K_B, M	pA_2	Slope of regression line	Correlation coefficient
Oxymetazoline	$(2.0\pm0.6)\times10^{-7}$ n = 9	3.5	6.5 ± 1.8				
Epinephrine	$(7.0\pm1.1)\times10^{-7}$ n = 12	1	10.5 ± 1.9				
Norepinephrine	$(1.2\pm0.2)\times10^{-6}$ n = 23	0.58	14.0 ± 2.3	$(1.9\pm0.9)\times10^{-8}$[a] n = 14	8.5[a]	0.77	0.99
				$(1.02\pm0.2)\times10^{-9}$[b] n = 24	8.8[b]	1.32	0.98
Phenylephrine	$(4.9\pm0.9)\times10^{-6}$ n = 7	0.14	7.4 ± 0.7				
Methoxamine	$(3.7\pm0.7)\times10^{-5}$ n = 14	0.019	9.6 ± 2.1				
Isoproterenol	$(2.0\pm0.5)\times10^{-4}$ n = 6	0.0035	10 ± 3.2				

EC_{50} = Agonist concentration producing half maximum contraction; E_{Am} = maximum contraction; K_B = dissocation constant for receptor-antagonist complex; pA_2 = intercept of regression line with abscissa in Schild plot. Significance levels (Student's t-test): $p<0.001$ between all consecutive pairs.

a) = tested with phentolamine as antagonist.
b) = tested with prazosin as antagonist.

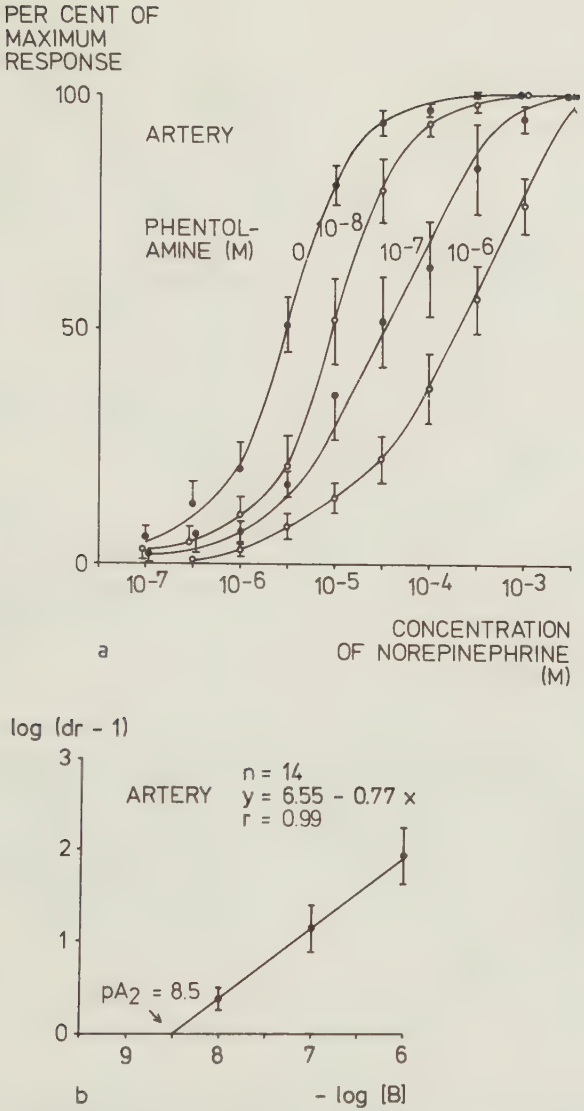


Fig. 4 Interaction between the α-receptor antagonist, phentolamine, and norepinephrine in mesenteric arteries. (a) Parallel shift in the concentration-response curve with increasing antagonist concentrations. Values are means, illustrated by different symbols for the different antagonist concentrations, + SEM of 5-7 vessels. (b) Schild plot based on 14 curve displacements tested on 5 arteries. The equation for the line is given, together with the correlation coefficient (r); dr = dose ratio; [B] = agonist concentration. The pA_2 value at the intercept of the line with the abscissa is indicated.

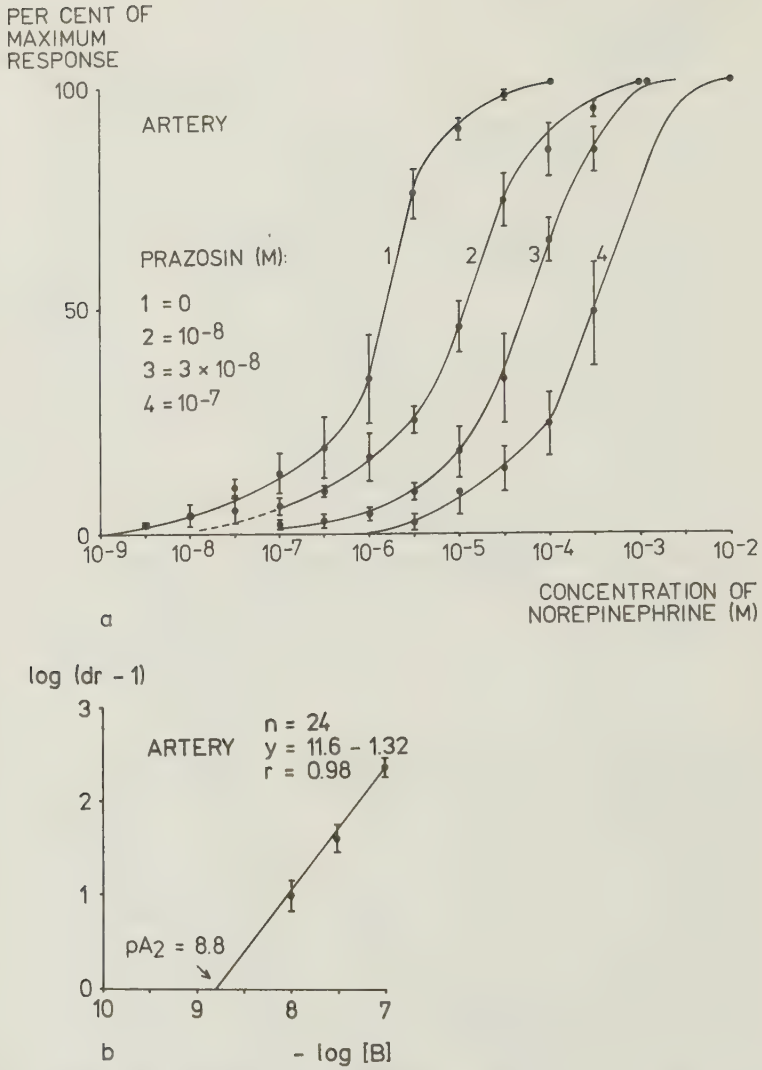


Fig. 5 Interaction between the α_1-receptor antagonist, prazosin, and norepinephrine in mesenteric arteries. (a) Parallel shift in the concentration-response curve with increasing antagonist concentrations. Values are means + SEM of 8-10 arteries. (b) Schild plot based on 24 curve displacements tested on 10 vessels. The equation for the line is given, together with the correlation coefficient (r); dr = dose ratio; [B] = agonist concentration. The pA_2 value at the intercept of the line with the abscissa is indicated.

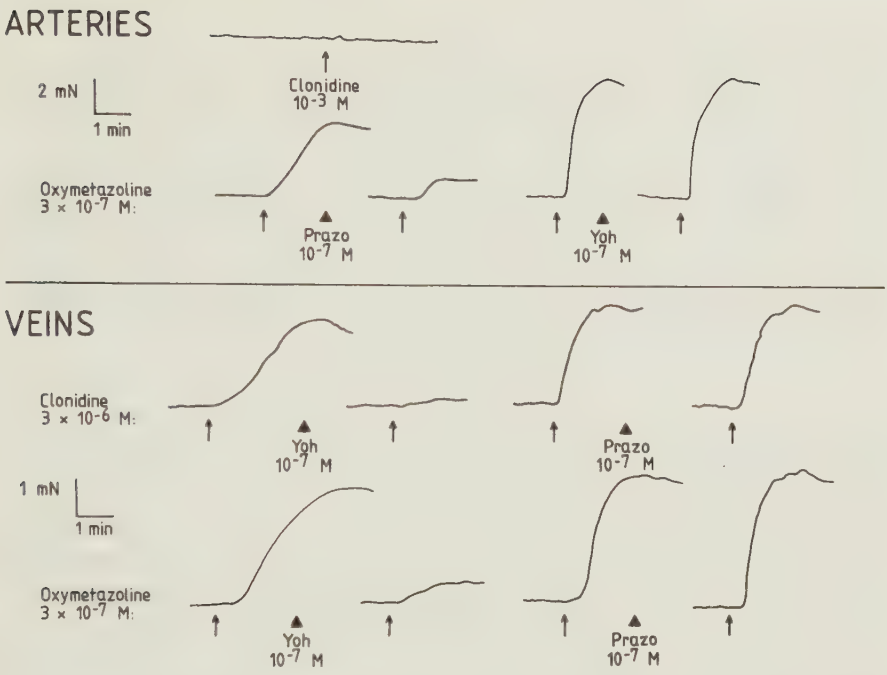


Fig. 6 Representative examples of reactions in mesenteric arteries and veins to clonidine and oxymetazoline (given at arrows), with or without prazosin (Prazo) or yohimbine (Yoh), given after previous wash-out 15-20 min prior to (and present throughout) next test with the agonist. In arteries, clonidine was without effect. The contraction induced by oxymetazoline was antagonized by 10^{-7} M prazosine, but not by the same dose of yohimbine. Calibration for contractile force (mN) and time (min) is shown.

Contractile Response in Mesenteric Veins

The potency rank for the sympathomimetic contraction of mesenteric veins (table III) differed from that of the arteries: epinephrine > clonidine > norephinephrine = phenylephrine $\geq$ oxymetazoline > methoxamine > isoproterenol. Ratios indicated by equality signs were not statistically significant ($p > 0.05$). The order of potency (between some of the standard agonists) indicate that the response in the veins, like in the arteries, is mediated by α-adrenergic receptors. This was confirmed by the mode of interaction of phentolamine with norepinephrine (fig.7).

Both yohimbine (fig. 8) and piperoxan (fig. 9) inhibited the norepinephrine-induced contraction in a competitive manner. The α_1-selective antagonist, prazosin, did not cause any parallel shift of the concentration-response curves in contrast to the situation for the arteries (cf. fig. 5). A detailed analysis of the E_{Am} values (fig. 10) showed that prazosin also did not reduce the maximum responses to norepinephrine (cf. Jauernig et al., 1978; Stevens and Moulds, 1981). In accordance with these observations clonidine contracted 6 of 7 veins tested, though the maximum was quite variable as it was also for oxymetazoline and methoxamine. The effects of both clonidine (3×10^{-6} M) and oxymetazoline (3×10^{-7} M), tested near their EC_{50} values, were inhibited by 10^{-7} M yohimbine but not by the same dose of prazosin (fig. 6). In view of these observations, it was notable that methoxamine contracted the mesenteric veins, since this compound is usually considered to be a selective agonist for the α_1-subtype of receptors. However, its relative potency was considered too low to warrant any further tests with antagonists. The degree of contractions and the quantitative effects of the antagonists tested are summarized in table III.

Table III. Pharmacological in vitro analysis of quantitative characteristics of adrenergic contractile mechanisms in human mesenteric veins responding to a particular agonist (mean ± SEM).

Amine tested	EC_{50}, M	Relative potency	E_{Am}, mN	K_B, M	pA_2	Slope of regression line	Correlation coefficient
Epinephrine	$(3.9\pm1.1)\times10^{-8}$ n = 4	1	6.2 ± 1.7				
Clonidine	$(1.7\pm0.4)\times10^{-7}$ n = 5	0.23	3.3 ± 1.3				
Norepinephrine	$(2.9\pm0.7)\times10^{-7}$ n = 27	0.13	9.5 ± 1.5	$(7.7\pm2.5)\times10^{-9a}$ n = 22	8.6	0.90	0.99
				$(5.3\pm1.0)\times10^{-8b}$ n = 37	7.6	0.96	0.99
				$(3.8\pm0.4)\times10^{-9c}$ n = 15	8.3	1.12	0.99
Phenylephrine	$(2.9\pm0.6)\times10^{-7}$ n = 8	0.13	2.6 ± 0.4				
Oxymetazoline	$(5.4\pm1.9)\times10^{-7}$ n = 8	0.08	1.5 ± 0.5				
Methoxamine	$(2.7\pm0.6)\times10^{-5}$ n = 8	0.0014	10.1 ± 0.2				
Isoproterenol	$(8.4\pm0.2)\times10^{-5}$ n = 7	0.00046	5.3 ± 1.7				

EC_{50}=Agonist concentration producing half maximum contraction; E_{Am}=maximum contraction; K_B= dissociation constant for receptor-antagonist complex; pA_2=intercept of regression line with abscissa in Schild plot. Significance levels (Student's t-test): epinephrine vs. clonidine, $p<0.05$; oxymetazoline vs. methoxamine, methoxamine vs. isoproterenol, $p<0.001$; others not significant.

a)=tested with phentolamine as antagonist; b)=tested with piperoxan as antagonist; c)=tested with yohimbine as antagonist.

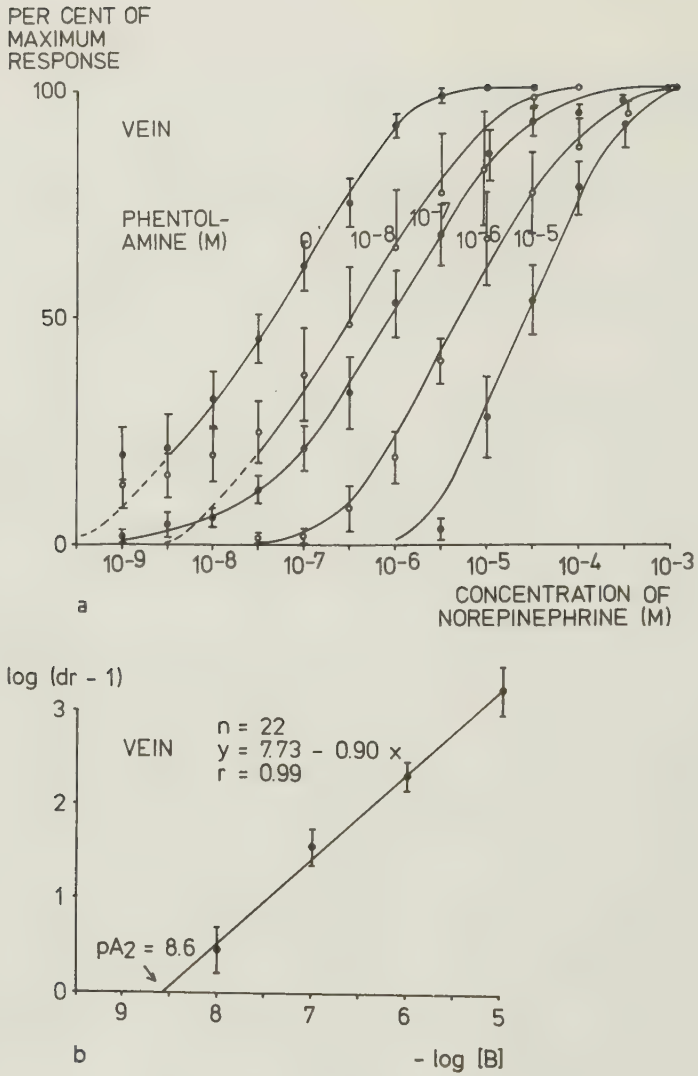


Fig. 7 Interaction between the α-receptor antagonist, phentholamine, and norepinephrine in mesenteric veins. (a) Parallel shift in the concentration-response curves with increasing antagonist concentrations. Values are means, illustrated with different symbols for the different antagonist concentrations, + SEM of 5-9 veins. (b) Schild plot based on 22 curve displacements tested on 7 vessels. The equation for the line is given, together with the correlation coefficient (r); dr = dose ratio; [B] = agonist concentration. The pA_2 value at the intercept of the line with the abscissa is indicated.

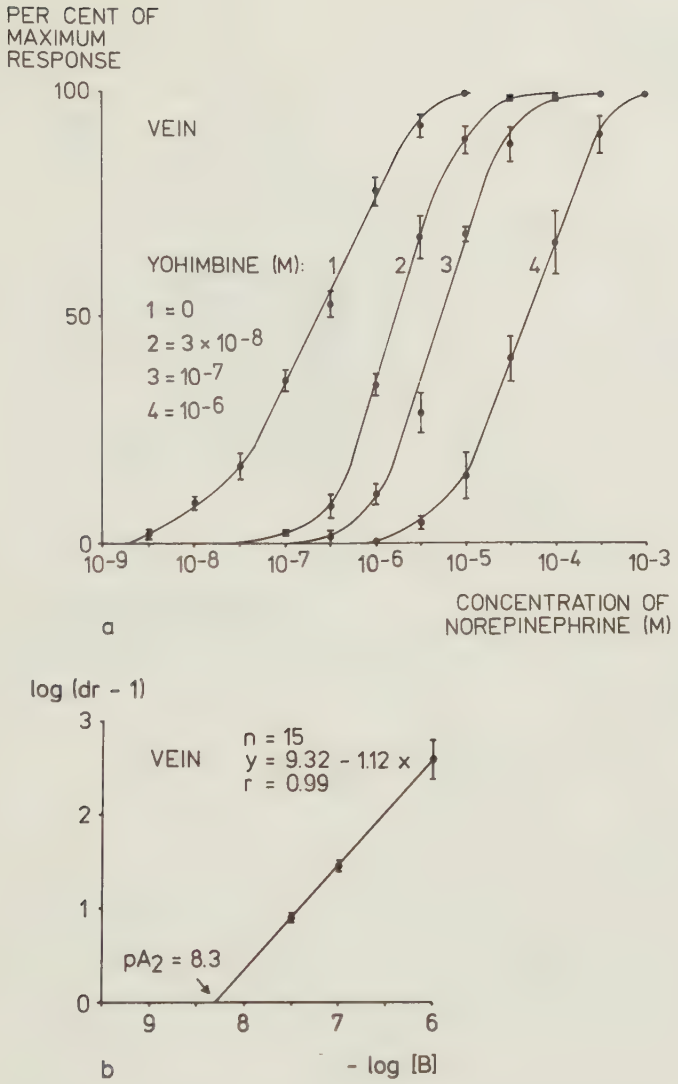
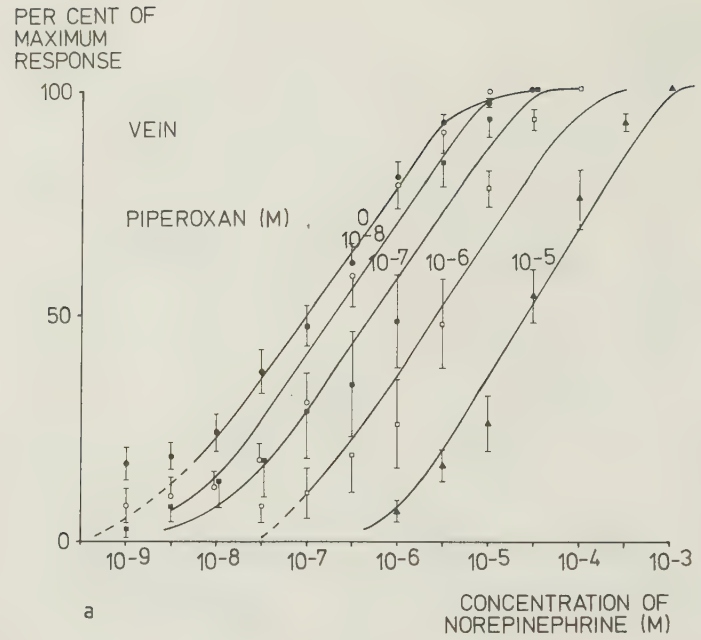


Fig. 8 Interaction between the α_2-receptor antagonist, yohimbine, and norepinephrine in mesenteric veins. (a) Parallel shift in the concentration-response curves with increasing antagonist concentrations. Values are means + SEM of 5 veins. (b) Schild plot based on 15 curve displacements tested on 5 vessels. The equation for the line is given, together with the correlation coefficient (r); dr = dose ratio; [B] = agonist concentration. The pA_2 value at the intercept of the line with the abscissa is indicated.



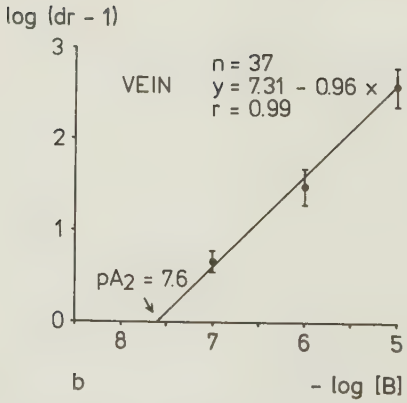


Fig. 9 Interaction between the α_2-receptor antagonist, piperoxan, and norepinephrine in mesenteric veins. (a) Parallel shift in the concentration-response curves with increasing antagonist concentrations. Values are means, illustrated with different symbols for the different antagonist concentrations, + SEM of 13-30 vessels. (b) Schild plot based on 37 curve displacements tested on 13 vessels. The equation for the line is given, together with the correlation coefficient (r); dr = dose ratio; [B] = agonist concentration. The pA_2 value at the intercept of the line with the abscissa is indicated.

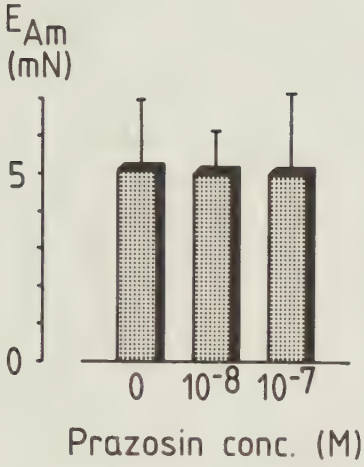


Fig. 10 E_{Am} for norepinephrine in mesenteric veins without or with prazosin. Values are means + SEM of 10 vessels. There was no statistically significant difference in the mean maximum contraction.

Dilatory Response

The dilatory response was tested in vessels given active tone by the addition of 10^{-6} to 10^{-5} M $PGF_2\alpha$. Phenoxybenzamine was added to the organ bath to a final concentration of 10^{-6} M in order to block any contractile effects of the sympathomimetic agonists tested. In other experiments potassium was used to create a contracture of the vessel before testing for any dilatory response. Neither isoproterenol nor terbutaline was able to cause any measurable dilatation in doses of 10^{-9} to 10^{-3} M tested on 30 arterial, and 16 venous preparations under conditions when both 10^{-10} M substance P and 10^{-11} M carbacholine dilated the vessels (fig. 11).

Fig. 11 Dilatory response of mesenteric arteries and veins given active tension by 3 x 10^{-6} M $PGF_2\alpha$ (PG). Dilatory agonists were tested in increasing molar (M) concentrations shown in their logarithmic forms at the arrows. The β-receptor agonist, isoproterenol (ISO), was without effect in either vessel under conditions when substance P (SP) induced marked dilatation. Calibration for contractile force (mN) and time (min) is shown.

Discussion

Most of the investigations on adrenergic receptors have been performed on tissues from laboratory animals. During the past decade an increasing interest has been focused on human blood vessels and their alterations in disease. Therefore, human material removed during surgery or autopsy have been more extensively used. Thus, a variety of human blood vessels have been examined pharmacologically in vitro (cf. Thulesius, 1983), including cerebral, pulmonary, coronary, digital, omental, and femoral arteries and veins, as well as the facial vein. The adrenergic receptor has not yet been isolated and characterized biochemically. Therefore, the classification of various receptor types has to a large extent been based on pharmacological procedures, according to which more or less specific adrenergic agonists and antagonists have been utilized to characterize the sympathomimetic response of a given tissue or organ. In this operational approach, similarities or differences in the response reflect similarities or differences in the types of receptors mediating the various responses (Furchgott, 1972).

Detailed pharmacological analysis on vascular adrenoceptors have generally been performed in vitro, and several different techniques (Moulds, 1983) have been applied for studying motor responses of isolated blood vessels. The spiral strip preparations give reproducible concentration-effect curves but suffer from the disadvantage of damaging the musculature, nerves, and endothelium which are all cut when the strips are prepared. Whole-mounted preparations of vascular segments for recording of circular motor activity represent another well-studied model. These ring preparations better reflect physiological conditions than the spiral strips because the morphology of the vessel wall remains intact and they primarily measure circular smooth muscle activity. The technique also allows for studies of vessels with smaller caliber than the spiral-cut technique.

In the present study, ring preparations (Nielsen and Owman, 1971; Edvinsson et al., 1974) from human mesenteric arteries and veins were used. For practical reasons during the surgical procedure, in an attempt to obtain fresh preparations, it was not found possible to prepare isolated vessels with a diameter of less than 0.5 mm. Although resistance to flow through the tissue is not primarily controlled by arteries of this large caliber (Folkow and Neil, 1971) their pharmacological response seems to be representative of that exhibited by arteries with a dimeter down to 0.1 mm

(e.g. MacKenzie et al., 1978). The relationship between initial tension and force of contraction has been studied in various types of blood vessels (Edvinsson et al., 1974; Högestätt et al., 1981). Characteristically, there is an optimum in the level of initial tension at which maximal contraction is developed by the agent applied. The amount of passive load presently chosen was well within the estimated maximum wall tension the vessel might be exposed to under in vivo conditions (without correcting for forces that may act on the vessel wall from the outside in situ).

The nature of the contractile response made it possible to use cumulative application of the agonists (van Rossum and van den Brink, 1963). Storage in a modified Krebs-Ringer solution at +4 ^{o}C for up to 2 days did not alter the dose-response relationship. In the course of the individual test periods of approximately 5 h, there was sometimes a decrease in E_{Am}, which was disclosed when control runs with agonists were compared at the beginning and at the end of the test periods. This was taken into consideration in the quantitative estimations. The EC_{50} values remained stable (cf. Rapoport and Bevan, 1983).

The order of potency by which the tested classical sympathomimetic amines (Ahlquist, 1948) induced a constriction of the vascular preparations showed that the response involved α-adrenoceptors (Furchgott, 1972). Pharmacological subdivision of α-adrenoceptors has been prompted by the demonstration also of prejunctional α-receptors. These α_2-types are found on sympathetic nerve endings, whereas the α-receptors located postjunctionally in the blood vessel wall appear to be constituted by a mixed population of α_1- and α_2-adrenoceptors (Starke, 1981; Langer and Shepperson, 1981). In the present study the distribution of α-receptor subtypes was found to differ in the human mesenteric arteries and veins.

The results from the blocking experiments indicate that the α_1 subtype of adrenergic receptor predominates in the mesenteric arteries: prazosin caused a parallel shift of the concentration-response curve towards higher norepinephrine levels, whereas yohimbine and piperoxan were without effect. In a corresponding way, the mesenteric veins seem to be equipped with mainly the α_2 subtype of receptors: both yohimbine and piperoxan inhibited the norepinephrine induced contraction in a competitive manner, whereas prazosin caused no parallel shift of the concentration-response curves. It has previously been found that prazosin markedly depresses the

maximal response to norepinephrine in human hand vessels, particularly metacarpal veins (Jauernig et al., 1978; Stevens and Moulds, 1981). In the human mesenteric veins, however, prazosin did not affect the E_{Am} value to any significant extent.

Prazosin has a high affinity for α_1-receptors with little or no affinity for presynaptic α-receptors (Cambridge and Greengrass, 1980). The pA_2 value obtained for the mesenteric arteries (8.8) agreed well with the corresponding values reported in the literature (8.2- 8.9) for a variety of smooth muscle tissues (for references, see Skärby et al., 1983). However, the slope of the regression line in the Schild plot was significantly higher than unity. This would suggest that the response to prazosin involved some other component in addition to the α_1-receptor antagonism, e.g. phosphodiesterase inhibition and/or a possible Ca^{2+} entry blocking action.

The pA_2 value for yohimbine on mesenteric veins (8.3) correlates best with the pA_2 values reported (7.36-8.32) for the interaction of this compound with α_2-receptors; the interaction of yohimbine with α_1-receptors has yielded pA_2-values that are considerably lower, ranging from 6.10 to 6.56 (for references, see Skärby et al., 1983). Piperoxan has a lower degree of selectivity for the α-receptor subtypes, although it has a preference for the α_2-type receptors (cf. Borowski et al., 1977; Timmermans et al., 1980; Doxey et al., 1983). It had, like yohimbine, no effect on the norepinephrine response on the arteries in the concentration range used in this work. However, it caused a parallel shift to the right, similar to yohimbine in mesenteric veins. The pA_2 value obtained (7.6) corresponded with its interaction with α-receptors in other smooth muscle tissues (Timmermanns et al, 1980; Doxey et al, 1983).

The relative potencies of agonists often agree with their relative affinity, provided the efficacies are equal (Starke, 1981). However, in the present material on human mesenteric vessels, there was usually a considerable variation in the E_{Am} values for the various agonists. Moreover, there is no obligatory relationship between affinity and efficacy, since the structural requirements for each of these parameters of drug action differ significantly (Ruffolo et al., 1980a, b). The use of agonists for pharmacological characterization of adrenergic receptor subtypes therefore porvided a much less reliable tool than the application of selective

antagonists. The response to clonidine agreed with the predominance of α_2- receptors in the mesenteric veins. It regularly contracted these vessels by an action that was inhibited by yohimbine but not by prazosin, and it had usually no effect on arteries in doses up to 10^{-3} M. On the other hand, the potency of phenylephrine was only slightly lower than that of clonidine in the veins. Our observations on the mesenteric arteries are consistent with similar findings on human omenterial arteries by Steen et al., (1984). The potency ratios for norepinephrine and phenylephrine in the arteries corresponded well with what could be expected for an α_1-adrenoceptor (Starke, 1981). It should be noted, though, that the relationship between these two agonists in the veins did not conform with an α_2-receptor.

The results with oxymetazoline and methoxamine are more difficult to interpret. Oxymetazoline acted only as a partial agonist on both arteries and veins. Although this compound is considered to be a strong α_2-agonist, it had a potency that was higher than any of the other agonists tested on the mesenteric arteries, and its effect on these was antagonized by prazosin (but not by yohimbine). In the veins it had a potency resembling that of phenylephrine (and norepinephrine). Here, the contraction was inhibited by yohimbine (but not by prazosin). Methoxamine, on the other hand, is often classified as a selective α_1-agonist. This amine had a low relative potency both in arteries and veins, and the EC_{50} values found in the two types of vessels were almost identical. Although oxymetazoline and methoxamine have been important in distinguishing between α_1- and α_2- receptor subtypes in central as well as several peripheral tissues (cf. Wikberg, 1979; Cambridge and Greengrass, 1980; Starke, 1981), they appear to be less useful in corresponding studies not only on human omental (Steen et al., 1983) and mesenteric arteries, but also in feline mesenteric arteries (Skärby et al., 1983).

Our conclusion about the nature of the contractile response favors the existence of primarily α_1-adrenoceptors in the mesenteric arteries, whereas the mesenteric veins are mainly equipped with α_2-adrenoceptors. The pharmacological analysis does not allow for any conclusion about the extent of concomitant presence of α_2- and α_1-receptors, respectively (or hitherto unknown adrenoceptor subtypes) in the arteries and veins.

The situation thus resembles observations made on human arteries and veins both from femoral and groin regions (Glusa and Markwardt, 1983) as well as from the omentum (Steen et al., 1983).

The catecholamines often exert strong vasodilatory effects, which may involve local activation of postjunctional β-adrenoceptors in the vessel wall (cf. Owman, 1980). Such vasodilatation is mediated in intracranial and coronary vessels by the β_1- receptor subtype, whereas vasodilatation in the peripheral vascular bed usually involves the β_2-subtype. In the human mesenteric arteries and veins, we were, however, not able to demonstrate any vasodilatory effects of the β-adrenoceptor agonists tested. This is not due to an inability of the vessels to respond by vasodilatation in vitro since carbamylcholine as well as substance P were found to produce extensive dilatation of both mesenteric arteries and veins.

During the last few years it has become evident that the endothelial cells covering the luminal surface of the blood vessels play a key role in the motor effects of certain vasoactive agents (Furchgott, 1981). The lack of dilatory effect of isoproterenol or terbutaline was apparently not due to a damaged vascular endothelium, since both substance P and carbacholine, which dilated the mesenteric vessels, require an intact endothelium for their effect (Furchgott, 1981). Moreover, the conditions under which the vessels were mounted in our organ bath do not damage the endothelium (unpublished observations) to a degree which would account for any loss of response mediated by these cells.

Acknowledgement: The authors thank Inga-Lill Bertilsson for skilful technical assistance.

References

Ahlquist, R.P.: A study of the adrenotropic receptors. Am. J. Physiol. 153: 586-600 (1948).

Arunlakshana, O.; Schild, H.O.: Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14: 48-58 (1959).

Baker, J.R.: Principles of biological microtechnique (Methuen, London 1970).

Borowski, E.; Starke, K.; Ehrl, H.; Endo, T.: A comparison of pre- and postsynaptic effects of α-adrenolytic drugs in the pulmonary artery of the rabbit. Neuroscience 2: 285-296 (1977).

Cambridge, D.D. Greengrass, P.M.: The pharmacology of antihypertensive drugs with special reference to vasodilators, α-adrenergic blocking agents and prazosin. Prog. Pharmacol. 3/4: 107-114 (1980).

Doxey, J.C.; Roach, A.G.; Smith, C.F.C.: Studies on RX 781094: a selective, potent and specific antagonist of α_2-adrenoceptors. Br. J. Pharmacol. 78: 489-505 (1983).

Edvinsson, L.; Nielsen, K.C.; Owman, C.: Influence of initial tension and changes in sensitivity during amine-induced contractions of pial arteries in vitro. Archs. int. Pharmacodyn. Thér. 208: 235-242 (1974).

Folkow, B.; Neil, E.: Circulation (Oxford University Press, London 1971).

Frank, O.: Die Elastizität der Blutgefässe. Z. Biol. 71: 255-272 (1920).

Furchgott, R.F.: The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory; in Blaschko, Muscholl, Handbook of experimental pharmacology, pp. 283-335 (Springer, Berlin, 1972).

Furchgott, R.F.: The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. Trends pharmacol. Sci. 2: 173-176 (1981).

Glusa, E.; Markwardt, F.: Characterization of postjunctional α-adrenoceptors in isolated human femoral veins and arteries. Arch. Pharmacol. 323: 101-105 (1983).

Högestätt, E.D.; Andersson, K.E.; Edvinsson, L.: Mechanical reactivity of rat middle cerebral and basilar arteries to various vasoactive agents recorded in vitro; in Cervós-Navarro, Fritschka, Cerebral microcirculation and metabolism, pp. 181-187 (Raven Press, New York 1981).

Iversen, L.L.: The uptake and storage of noradrenaline in sympathetic nerves (Cambridge University Press, Cambridge 1967).

Jauernig, R.A.; Moulds, R.F.W.; Shaw, J.: The action of prazosin in human vascular preparations. Archs. int. Pharmacodyn. Thér. 231:81-89 (1978).

Langer, S.Z.; Shepperson, N.B.: Subclassification of α-adrenoceptors into α_1- and α_2-categories: relevance to antihypertensive therapy; in Worcel, New trends in arterial hypertension. Inserm Symp. No. 17, pp. 73-85 (Elsevier, North Holland Biomedical Press, Amsterdam, 1981).

Lillehei, R.C.; Washington, D.C.: The intestinal factor in irreversible hemorrhagic chock. Surgery, St. Louis, 42: 1043-1054 (1957).

MacKenzie, E.T.; Stewart, M.; Young, A.R.; Harper, A.M.: Cerebrovascular actions of serotonin: in Cervós-Navarro, Betz, Ebhardt, Ferszt, Wüllenweber, Pathology of cerebrospinal microcirculation, pp. 77-83 (Raven Press, New York 1978)

Moulds, R.F.W.: Techniques for testing isolated blood vessels. Gen. Pharmacol. 14: 47-53 (1983).

Nielsen, K.C.; Owman, C.: Contractile response and amine receptor mechanisms in isolated middle cerebral artery of the cat. Brain Res. 27: 33-42 (1971).

Owman, C.: Vascular autonomic nerves and corresponding receptors, with particular reference to neurogenic mechanisms of vasodilatation. Prog. Pharmacol. 3: 47-68 (1980).

Rapoport, R.M.; Bevan, J.A.: Effect of contraction on the subsequent responsiveness and maximum contractility of the rabbit ear artery and saphenous vein in vitro. Blood Vessels 20: 44-55 (1983).

Ruffalo, R.R.; Wadell, J.E.; Yaden, E.L.: Receptor interactions of imidazolines. IV. Structural requirements for alpha-adrenergic receptor occupation and receptor activation by clonidine and a series of structural analogues in rat aorta. J. Pharmac. exp. Ther. 213: 267-272 (1980a).

Ruffalo, R.R.; Yaden, E.L.; Wadell, J.E.: Receptor interactions of imidazolines. V. Clonidine differentiates postsynaptic alpha-adrenergic receptor subtypes in tissues from the rat. J. Pharmac. exp. Ther. 213: 557-561 (1980b).

Rossum, J.M. van; Brink, F.G. van den: Cumulative dose-response curves. I. Introduction to the technique. Archs. int. Pharmacodyn. Thér. 143: 240-246 (1963).

Samnegård, H.; Tydén, G.; Thulin, L.; Friman, L.; Udén, R.: Effect of somatostatin on regional splanchnic blood flows in man. Angiographic studies. Acta chir. scand., suppl. 500; pp. 71-73 (1980).

Schild, H.O.: The use of drug antagonists for identification and classification of drugs. Br. J. Pharmacol. 2: 251-258 (1947).

Skärby, T.; Andersson, K.-E.; Edvinsson, L.: Pharmacological characterization of isolated feline cerebral and peripheral arteries. Acta physiol. scand. 117: 63-73 (1983).

Starke, K: α-Adrenoceptor subclassification. Rev. Physiol. Biochem. Pharmacol. 88: 199-236 (1981).

Steen, S.; Skärby, T.V.C.; Norgren, L.; Andersson, K.-E.: Pharmacological characterization of postjunctional α-adrenoceptors in isolated human omental arteries and veins. Acta physiol. scand. 120: 109-116 (1983).

Stephenson, R.P.: A modification of receptor theory. Br. J. Pharmacol. 11: 379-393 (1956).

Stevens, M.J.; Moulds, R.F.W.: Heterogeneity of postjunctional α-adrenoceptors in human vascular smooth muscle. Archs. int. Pharmacodyn. Thér. 254: 43-47 (1981).

Thulesius, O.: First international symposium on pharmacology of human blood vessels. Gen. Pharmacol. 14: 1-208 (1983).

Timmermans, P.B.M.W.M.; Meel, J.C.A. van; Zwieten, P.A. van: Evaluation of the selectivity of α-adrenoceptor blocking drugs for postsynaptic α_1- and α_2-adrenoceptors in a simple animal model. J. Auton. Pharmacol. 1: 53-60 (1980).

Törnebrandt, K.; Nobin, A.; Ericsson, M.; Thomson, D.: Circulation, respiration and serotonin levels in carcinoid patients during neurolept anaesthesia. Anaesthesia 38: 957-967 (1983).

Wikberg, J.E.S.: The pharmacological classification of adrenergic α_1- and α_2-receptors and their mechanisms of action. Acta physiol. scand., suppl. 468, p. 1 (1979).

Serotonergic Smooth Muscle Receptors in Human Mesenteric Arteries and Veins

Kenneth Törnebrandt*, Anders Nobin• and Christer Owman°

Departments of Anesthesiology*, Surgery•, and Histology°, University of Lund, S-221 85 LUND, Sweden

Running title: Serotonergic receptors in mesenteric vessels

Acknowledgements of support: The study was supported by grants from the Swedish Medical Research Council (grants No 12X-712 and 14X-732), Janssen Leo Pharma AB

Proofs and correspondence to: Kenneth Törnebrandt, M.D., Department of Anesthesiology, University of Lund, S-221 85 LUND, Sweden

Abstract

Isolated human mesenteric arteries (diameter less than 1 mm) and veins (diameter less than 2 mm) were tested in vitro for their contractile and dilator responses to 5-hydroxytryptamine (5-HT) under standardized conditions. This allowed for quantitative estimation of various receptor characteristics: relative agonist potency, concentration for half maximum response (EC_{50}), amount of maximum response (E_{Am}), and affinity of antagonist to receptor (K_B and pA_2). 5-HT caused concentration-dependent contractions of the mesenteric vessels. The arteries were more sensitive to 5-HT than to norepinephrine (NE), as previously tested, whereas in the veins the sensitivity to 5-HT and NE was similar. The $5\text{-}HT_2$ receptor antagonist, ketanserin, caused a competitive, partly irreversible inhibition of the 5-HT induced contraction. The blockade by the α-antagonist phenotalmine, on the other hand, was not competitive. 5-HT did not produce any vasodilatory effects in the vessels tested after previous contraction (with, e.g., prostaglandin $F_{2\alpha}$) and under conditions of an intact endothelium. In low concentrations (tested below EC_{50}) both 5-HT and NE potentiated each other's contractile effects in the arteries, but not in the veins.

Key words: Human mesenteric arteries and veins, smooth muscle contraction in vitro, quantitative receptor pharmacology, 5-hydroxytryptamine, receptor potentiation, ketanserin, R 55 667.

Introduction

The physiological role of 5-hydroxytryptamine (5-HT) in the cardiovascular system, and its potential importance in pathologic conditions is debated. This is not surprising, since the amine not only stimulates or inhibits the function of a variety of smooth muscles and autonomic nerves but can, in addition, induce heterogenous responses which differ not only between species, but also between animals of the same species, and even in successive tests in a given individual (Essman, 1977-78; Douglas, 1980). Another barrier to our understanding of the role of 5-HT is lack of knowledge regarding the different classes of 5-HT receptor types mediating the responses (Humphrey, 1983). In the periphery, 5-HT affects not only the cardiovascular system, but also respiratory and gastrointestinal functions (Douglas, 1980).

Studies on vascular smooth muscle cells have elucidated that 5-HT causes contraction of most isolated vascular preparations (Shepherd and Vanhoutte, 1979; Edvinsson et al., 1978; van Nueten et al., 1981; Arneklo-Nobin et al., 1985). The contraction may be due to direct activation of serotonergic receptors on the vascular endothelial or smooth muscle cells, amplification of the response to other vasoconstrictor agents, activation of α-adrenoceptors in the vascular wall, or displacement of endogenous norepinephrine (NE) from adrenergic nerve endings with subsequent activation of α-adrenoceptors pre- or postjunctionally.

Vasodilator effects of 5-HT have mainly been observed in the intact organism, although certain isolated vascular preparations relax when exposed to 5-HT (Edvinsson et al., 1978; Vanhoutte, 1978; Vanhoutte et al., 1981; Mecca and Webb, 1984; Cohen et al., 1983). The dilatation caused by 5-HT may be due to direct serotonergic activation of endothelial or smooth muscle receptors, inhibition of neurogenic vasoconstrictor release, activation of α-adrenoceptors, or an activation of postjunctional inhibitory receptors, with yet unknown pharmacological characteristics.

Enterochromaffin cells and platelets are well-known sources for 5-HT. In addition, 5-HT has been demonstrated within nerves in the central nervous system not only in laboratory animals (Dahlström and Fuxe, 1965; Steinbusch, 1984), but also in man (Nobin and Björklund, 1973). There is recent immunohistochemical evidence that 5-HT is stored in peripheral nerves, also in man, as demonstrated in the gut (Costa et al., 1982; Griffith and Burnstock, 1983) and around intracranial blood vessels (Griffith and Burnstock, 1983).

There is much evidence to suggest that 5-HT is involved in the etiology of a varity of disorders due to altered vascular function such as migraine, stroke, the Raynaud-phenomenon, and the carcinoid syndrome (cf. De Clerck and Vanhoutte, 1982).

The present study was undertaken to clarify postjunctional serotonergic vascular effects utilizing human mesenteric blood vessels as model. The investigation is part of an attempt to elucidate the pathophysiological basis for cardiovascular changes in patients with extended carcinoid tumor growth associated with high circulating levels of 5-HT.

Material and Methods

Material

Small segments of mesenteric arteries and veins were obtained from approximately 60 healthy men and women, aged 20-70 years, undergoing surgery due to non-endocrine ventricular or intestinal malignancy. Morphine chloride and scopolamine bromide, or droperidol and fentanyl, were given as preanesthetic medication. Anesthesia was induced by pentothal sodium, or droperidol together with fentanyl administered intravenously. Intubation was facilitated by succinylcholine and the patients were ventilated with dinitrous oxide and oxygen. Supplementary doses of pancuronium bromide and fentanyl were given as needed. The patients were informed about the purpose of the investigation and had given their consent to the excision of the vascular prepartions.

Vascular preparations

Arteries (0.4-0.9 mm diameter) and veins (0.5-1.7 mm diameter) were dissected from terminal branches of the mesenteric vessels close to the intestines. The vessels were removed with an atraumatic technique and the ischemic period was less than 30 sec. They were then immediately transferred to a cold, modified Krebs-Ringer solution (for composition, see below) and kept at +4 °C in a refrigerator until the experiments were performed on the same or the next day. A 5-mm long piece of the vessel was prepared free from surrounding connective tissue and mounted between two L-formed metal holders in a 5-ml organ bath for recording of circular motor activity. The organ bath contained a modified Krebs-Ringer solution of the following composition (mM concentration): NaCl 118; KCl 4.5; $CaCl_2$ x 2 H_2O 2.5; $MgSO_4$ x 7 H_2O 1.0; $NaHCO_3$ 25; KH_2PO_4 1.0; glucose 6.0. The temperature in the bath was thermostatically maintained at +37 °C and the

buffer solution was continuously aerated with a mixture of 95% O_2 and 5% CO_2 giving pH 7.40 (range 7.35-7.45). The contractile and dilator responses were recorded isometrically with Grass FT03C force-displacement transducers and monitored on a Grass 79D polygraph.

The contractile response of mesenteric vessels to NE has previously been evaluated with regard to the influence of initial passive tension given to the vessels (Törnebrandt et al., 1985). In accordance with these results an initial load of 20-35 mN were applied to the vessels, followed by a 60-90 min accommodation period before the tests with agonists and antagonists were started.

In vitro recordings

In a pilot series of experiments, changes in the sensitivity to 5-HT were studied in one and the same vessel used either 1-2 h after operation or after storage for 24 h in a buffer solution at +4 °C. The response to 5-HT was also tested over a period of several hours in order to evaluate the reproducibility and the degree of tachyphylaxis.

In the main series of experiments the contractile and dilatory responses to various agonists and antagonists were tested. 5-HT was added to the bath in a cumulative way (van Rossum and van den Brink, 1963) in order to obtain full concentration-response curves since pilot experiments had shown that fractionated application facilitated tachyphylaxis. When contractile activity was tested, 10^{-6} M propranolol was present in the bath in order to block any β-adrenoceptor mediated dilatory effects (cf. Edvinsson et al., 1978). Several concentrations of various antagonists, each followed by a new concentration-response curve for the agonist, were administered for the quantitative characterization of the smooth muscle receptors involved (Furchgott, 1972). When dilatory responses were studied, the vessels were given an active tone by 10^{-7}-10^{-6} M of either prostaglandin $F_{2\alpha}$($PGF_{2\alpha}$), uridine triphosphate (UTP), or by 124 mM potassium. The contraction was found to remain at a steady level for at least 30 min. During that time varying concentrations of agonists were added.

The interaction between NE and 5-HT was studied in separate experiments in which the contractile effect of NE was tested in the presence of submaximal doses of 5-HT and vice versa.

Drugs

The following compounds were tested: L-arterenol hydrochloride, 5-hydroxytryptamine creatinine sulphate (Sigma), carbamylcholine chloride (Aldrich), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Amoglandin, Recip), uridine triphosphate (UTP; Sigma), phentolamine (Regitin, CIBA-Geigy), ketanserin tartrate and R 55 667 (Janssen), propranolol hydrochloride (Inderal, ICI), phenoxybenzamine hydrochloride and substance P (Sigma). The substances were dissolved in 0.9% saline. The catecholamine solutions contained 0.2 mg/ml ascorbic acid to minimize oxidation. The concentrations below are given as final molar concentration in the bath.

Statistics

Student´s t-test for unpaired and paired observations was used to compare mean values. Regression lines were obtained by the method of least squares.

Results

General mechanical properties

The contractile effects of 5-HT were identical whether the vessel was tested shortly after surgical removal or on the following day (Fig. 1a). Cumulative and fractionated concentration-response curves to 5-HT were similar, though not identical (Fig. 1b). This was due to a greater varia-

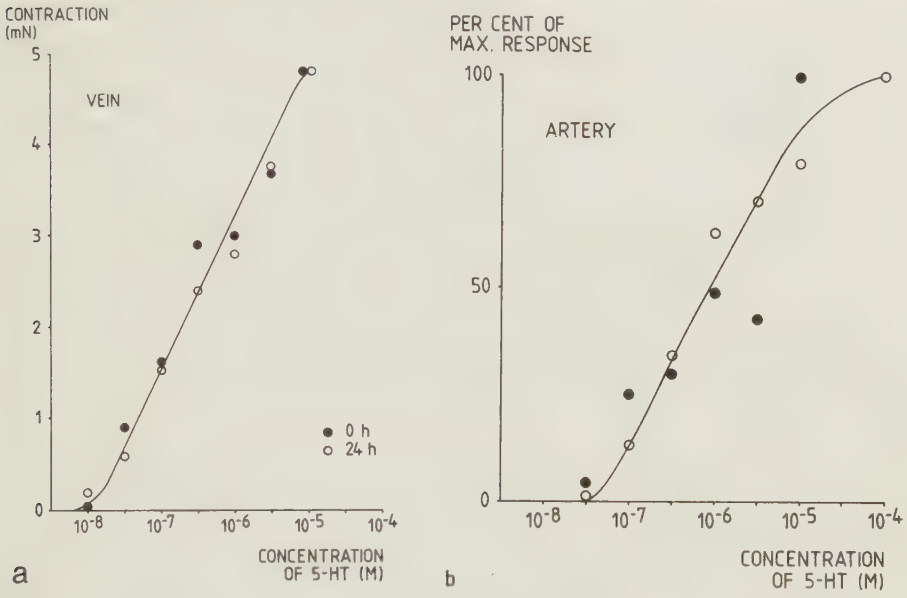


Fig. 1. a 5-HT-induced contraction into different segments of mesenteric artery from the same patient tested immediately after surgical removal (●) and after 24 h storage in buffer solution at +4 °C (○). Storage did not affect the response. b Contractile response to 5-HT into segments of mesenteric artery following administration by either cumulative application (○) or fractionated application (●). In the latter case the agonist was washed out between each single, increasing concentration. A greater variability in the response, including a varying degree of tachyphylaxis, was seen in the case of fractionated application.

bility in the response following fractionated application of 5-HT, and it also included a varying degree of tachyphylaxis. Since the tachyphylaxis was inconsistent there was no significant change in either E_{Am} (maximum contraction) or EC_{50} (agonist concentration at half maximum contraction) as evaluated from several full concentration-reponse curves obtained in the course of 4-5 hrs (Fig. 1c).

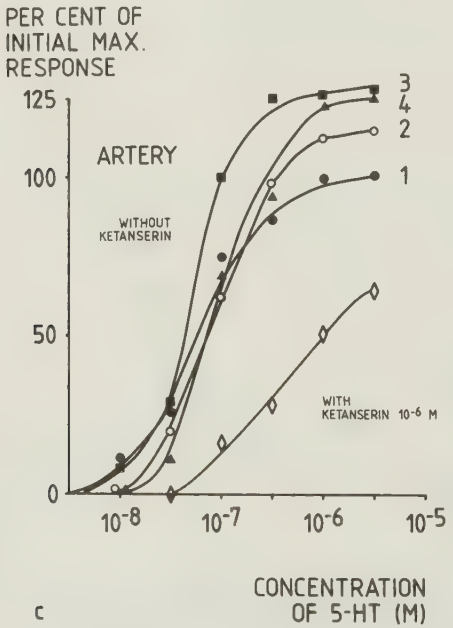


Fig. 1. c Repeated full concentration-response curves to 5-HT alone (curves 1 to 4) or in the presence of 10^{-6} M ketanserin. Contractile response is expressed as per cent of the first test, each value being the mean of 4 segments from the same mesenteric artery. There was no significant changes in either E_{Am} or EC_{50} that could be attributed to tachyphylaxis, whereas ketanserin significantly inhibited the contraction.

Contractile response

5-HT induced contraction of both mesenteric arteries and veins in a concentration-dependent manner (Fig. 1c, 3 and 5) with mean (± SEM) E_{Am} values of 16.4±3.0 mN and 16.4±3.1 mN, respectively. Ketanserin in increasing concentrations produced a dextral shift of the concentration-response curve for 5-HT (Fig. 2a and c). However, the curves did not have a simple sigmoid shape. For both types of vessels an increasing degree of deformation occurred already at antagonist concentrations above $3x10^{-8}$ M, so that only concentrations higher than the EC_{50} levels were included in the parallel shift of the curve. This was confirmed in the Schild plots (Arunlakshana and Schild, 1959) in which the values fitted a straight line (correlation coefficients, $r_{artery} = 0.81$ and $r_{vein} = 0.73$) with a slope of 0.85 for the arteries and 1.02 for the veins (Fig. 2b and d). This was in neither case significantly different from unity ($p>0.05$). The intercept of the line with the abscissa (pA_2) was 8.5 and 8.2 for arteries and veins, respectively. The corresponding mean values for the receptor-antagonist complex, expressed as the K_B value (Furchgott, 1972) for ketanserin, were (9.2±3.0) $x10^{-9}$ M in the arteries and (1.3±5.4) $x10^{-8}$ M in the veins.

In addition to the above-mentioned component of a parallel shift there was also a tendency to a reduced slope in the concentration-response curves, particularly evident at low 5-HT levels in the presence of the higher ketanserin concentrations. This antagonistic action of ketanserin was clearly distinguishable, including the progressive depression the E_{Am} values, from any tachyphylaxic effect of 5-HT (Fig. 1c).

The other 5-HT antagonist tested, R 55 667, had no effect on the EC_{50} values for the indole, but there was a progressive reduction in the E_{Am} value obtained in the consecutive concentration-response curves. This is illustrated in Fig. 3, also showing that the blockade was very prominent at 10^{-8} M in the veins (Fig. 3b).

In the case of both ketanserin and R 55 667 the reduced E_{Am} value obtained in a given concentration-response experiment remained largely unchanged despite repeated washes.

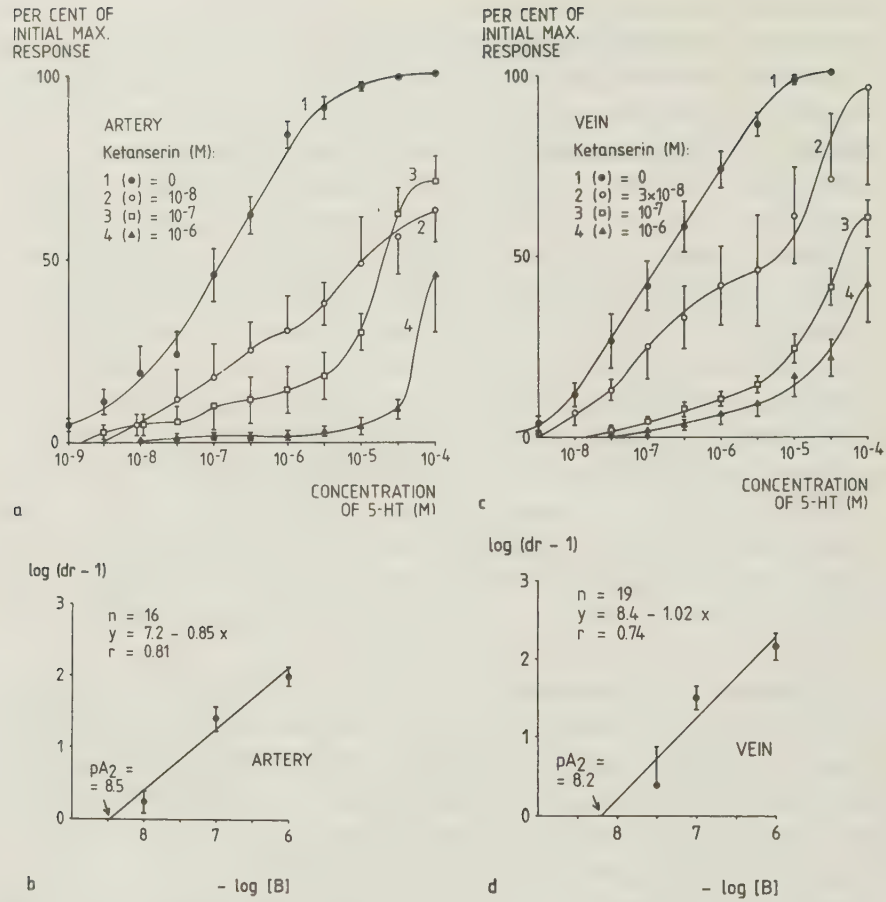


Fig. 2. Interaction between the serotonergic antagonist, ketanserin, and 5-HT in mesenteric vessels. a Arteries. Heterogenous inhibition (see text) of the concentration-response curves with increasing antagonist concentrations. Values are means + SEM of 3-14 vessels. The initial maximum response was set as 100 per cent, and the subsequent values obtained was related to this initial response. b Arteries. Schild plot based on 16 curve displacements (only components above EC_{50} used for calculation; see text) tested on 12 arteries. c Veins. Heterogenous inhibition of the concentration-response curves with increasing antagonist concentrations. Values are means + SEM of 4-14 vessels. The initial maximum response was set as 100 per cent, and the subsequent values obtained was related to this initial response. d Veins. Schild plot based on upper portions of 19 curve displacements tested on 12 veins. The equation for the lines are given in b and d, together with the correlation coefficients (r); dr=dose ratio, [B]=agonist concentration. The pA_2 values at the intercept of the lines with the abscissa are indicated.

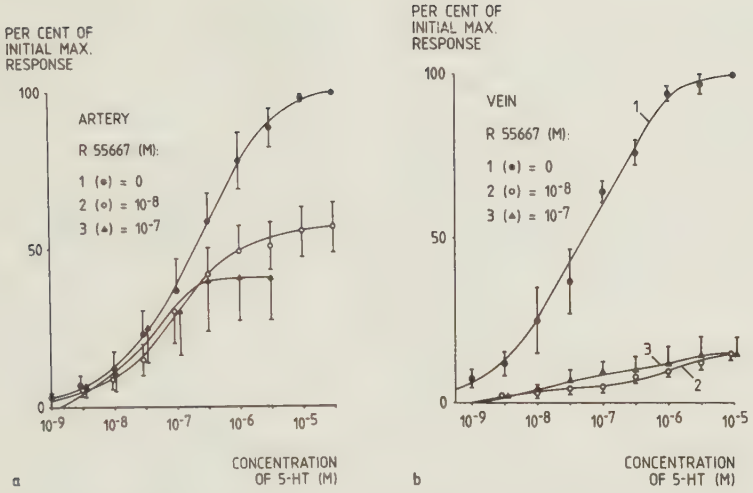


Fig. 3. Interaction between the serotonergic antagonist, R 55 667, and 5-HT in mesenteric arteries a and veins b. Values are means ± SEM of 8-10 arteries and 4 veins. The EC_{50} values were uneffected while the E_{Am} values were progressively reduced, especially in the veins.

The NE induced α-adrenergic contraction of human mesenteric arteries and veins (Törnebrandt et al., 1985) was not affected by either ketanserin (10^{-9}-10^{-6} M) or R 55 667 (10^{-9}-10^{-7} M). In Fig. 4 this is illustrated for

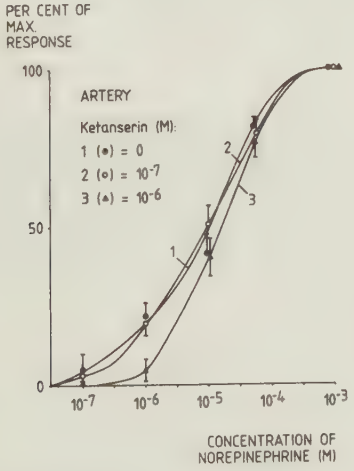


Fig. 4. Concentration-response curves to norepinephrine alone and in the presence of the serotonergic antagonist, ketanserin. Values are means ± SEM of 4 vessels. The contraction was not affected by ketanserin.

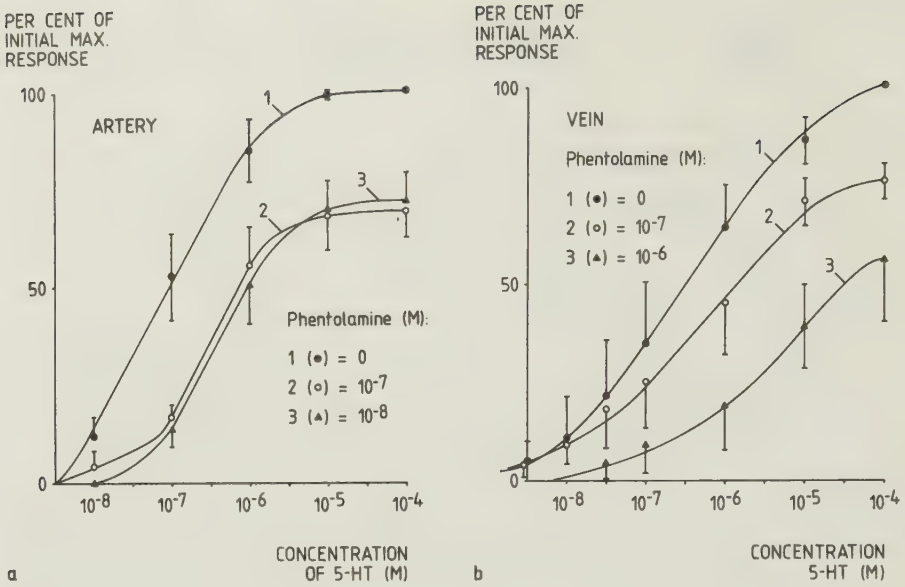


Fig. 5. Interaction between the α-receptor antagonist, phentolamine, and 5-HT in mesenteric arteries a and veins b. Values are means ± SEM of 4-9 arteries and 4-7 veins. In both types of vessels, phentolamine reduced the E_{Am} values in increasing concentrations, and it also tended to shift the EC_{50} values towards higher 5-HT concentrations, though this effect was inconsistent.

mesenteric arteries in the case of ketanserin. The effect of phentolamine on the 5-HT response was tested in both arteries and veins (Fig. 5). In increasing concentrations the α-antagonist reduced the E_{Am} value and also tended to increase EC_{50} towards higher 5-HT concentrations. However, this latter effect was not consistent and did not allow for the construction of Schild plots.

The interaction between 5-HT and NE on the vascular smooth musculature was tested in separate experiments. The contractile response to either of the amines was first recorded alone (in low concentrations, up to EC_{50}) and then in the presence of the other agonist, so that the potentiating effect of both 5-HT and NE could be elucidated. It can be seen from the typical examples illustrated in Fig. 6, which also includes the mean values (± SEM) for the contractions, that both 5-HT and NE markedly potentiated each other's contractile action in mesenteric arteries but not in veins.

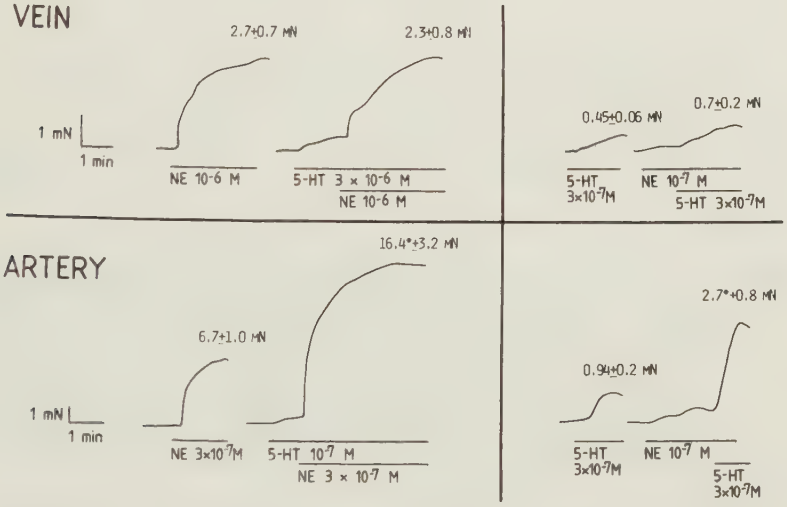


Fig. 6. Interaction between 5-HT and norepinephrine (NE) in mesenteric vessels. Typical examples are shown together with mean values ± SEM of 7 arteries and 8 veins. In the arteries, but not in veins, both 5-HT and NE markedly potentiated each other's contractile actions.

Dilatory response

The dilatory response was tested in 30 mesenteric arteries and 16 veins pre-contracted by $PGF_{2\alpha}$, UTP, or high potassium concentration. Phenoxybenzamine (10^{-6} M) was present in the organ bath to block any contractile effects of the sympathomimetic agonists tested. 5-HT was without dilatory effect in the 10^{-9} -10^{-4} M concentrations tested. Nor did isoproterenol or terbutaline cause any measureable dilatation in concentrations of 10^{-9} to 10^{-3} M, under conditions when either 10^{-10} M substance P or 10^{-11} M carbacholine dilated the vessels (Fig. 7).

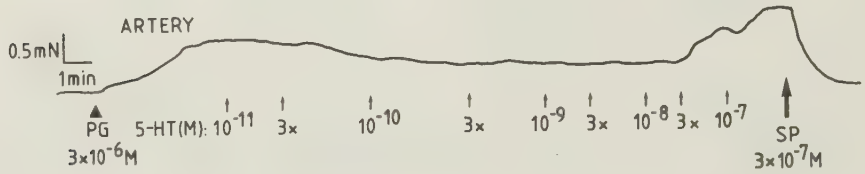


Fig. 7. Representative test of dilatory response in mesenteric artery (in the presence of 10^{-6} M phenoxybenzamine to block α-adrenoceptors) given active tension by $3x10^{-6}$ M prostaglandin (PG) $F_{2\alpha}$. The effect of 5-HT was tested in increasing molar (M) concentrations and compared to that of $3x10^{-7}$ M substance P (SP). The lack of dilatory effect of 5-HT could be clearly distinguished from the characteristic slight sagging of the PG-induced tone, whereas at the highest concentration 5-HT enhanced the contraction, from which SP efficiently dilated the vessels.

Discussion

The present study has demonstrated that 5-HT induces concentration-dependent contraction of isolated human mesenteric arteries and veins. To judge from the EC_{50} values the intrinsic activity was similar for both types of vessels and comparable to those obtained for human digital and intracranial arteries and veins (Edvinsson et al., 1978, 1984; Arneklo-Nobin and Owman, 1985). In the mesenteric arteries the contractile response to 5-HT was more pronounced than that to NE, whereas for the veins the sensitivity was similar (Törnebrandt et al., 1985). Our results thus demonstrate that isolated human mesenteric vessels respond to 5-HT in a pattern comparable to that shown for isolated vessels from laboratory animals (van Nueten et al., 1981).

5-HT has also been shown to display potent vasodilator properties (McCubbin et al., 1962; Walsh, 1967; McGrath et al., 1977; Mecca and Webb, 1984). These observations have mainly been based on experiments in the intact organism, particularly at the level of the small arteries (Mac Kenzie et al., 1978), although certain isolated vascular preparations may relax when exposed to this indole (Edvinsson et al., 1978). Against this background it has been proposed that in a given vascular bed, the net effect of 5-HT is determined by the balance between the actual constrictor and dilator properties of the substance (van Nueten, 1982).

It has recently been demonstrated that the 5-HT induced vasodilatation is dependent on an intact endothelium (Cocks and Angus, 1983; Cohen et al., 1983). This vascular relaxation is antagonized by methysergide but not by ketanserin. In view of these observations it is obvious that also the endothelium mediated effect has to be included in the net response to the contractile and dilatory effects of 5-HT. It has been proposed that the serotonergic receptors on the endothelial cells may be of a different subtype than those mediating direct contraction of smooth muscle cells (Cohen et al., 1983). In the present study 5-HT failed to dilate human mesenteric vessels previously contracted with $PGF_{2\alpha}$, UTP, or high potassium concentration. This may be due to regional differences or species variation in the dilatory effect of 5-HT, or to the fairly large vessel calibre presently used responding to 5-HT only by constriction, in contrast to vessels of smaller size (Mac Kenzie et al., 1978). The lack of dilatory response to 5-HT was not related to a damaged endothelium since the vessels dilated well in the presence of substance P or carbacholine, which both require an intact endothelium for their dilatory action (Furchgott, 1981).

Another aspect of the role of the endothelium in vascular reactions has been presented by Cocks and Angus (1983). They showed that NE and 5-HT were significantly more powerful vasoconstrictors in the absence of the endothelium in experiments on isolated coronary arteries from pigs and dogs. It was suggested that the constrictor amines release a vasodilator substance from endothelial cells that can act as a physiological antagonist of the direct contractile response of the smooth musculature.

The contractile response in the human mesenteric arteries and veins to 5-HT appeared to be mediated by serotonergic receptors since it was inhibited by ketanserin but was not affected by the α-receptor antagonist, phentolamine. This corroborates previous observations that contractile responses to 5-HT in various vessel preparations (e.g. rat caudal artery, canine gastroepiploic artery and human hand arteries) are antagonized in a competitive fashion by low doses of ketanserin in vitro (van Nueten et al., 1981; Arneklo-Nobin and Owman, 1985). Ketanserin is regarded to be a specific antagonist for the 5-HT_2 type of receptor (van Nueten et al., 1981), which is supported by receptor binding studies (Leysen et al., 1981). It has a high degree of specificity also in the sence that it is only a weak antagonist of adrenergic α-receptors (van Nueten et al., 1981). In contrast to most other 5-HT antagonists, ketanserin is devoid of 5-HT-like agonistic properties and it is not recognized by the 5-HT_1 binding sites (Leysen et al., 1981; van Nueten et al., 1981).

In both human mesenteric arteries and veins, the antagonism of the 5-HT induced contraction by ketanserin involved not only a shift of the EC_{50}-values towards higher agonist concentrations, but also a decrease in the E_{Am}. The results indicate a competitive inhibition, secured in Schild plots, with a component of irreversible receptor blockade, thus resembling the situation recently observed for human hand arteries (Arneklo-Nobin and Owman, 1985). Another, better known, example of "irreversible competitive antagonism" is the blockade of α-adrenoceptors by phenoxybenzamine and other β-haloalkylamines (Furchgott, 1966). The pA_2 value obtained in both types of vessels agree with those previously reported for human hand arteries and veins (Arneklo-Nobin and Owman, 1985).

The antagonist, R 55 667, has been introduced as a 5-HT_2 receptor blocker with higher affinity and less α-receptor antagonistic action than ketanserin (Paul Janssen, personal communication). In our experiments the pronounced blockade of the 5-HT induced contraction did not reveal any competitive features, contrarily to the action of ketanserin, the marked

depression of the E_{Am} possibly reflecting a particularly high affinity for the receptor and therefore unsurmountable by 5-HT.

The interrelation, including possible identity, between the 5-HT receptors and the α-adrenoceptors in vascular tissues has attracted considerable interest. The 5-HT induced contraction in the dog saphenous vein is blocked by α-adrenergic antagonists (Humphrey, 1978), and it is claimed that the effects of ketanserin in lowering blood pressure in animals is mediated via an α-adrenoceptor blockade (van Nueten et al., 1981). Our results, however, distinctly support the assumption of separate 5-HT and α-adrenoceptors in human mesenteric vessels. The potent vasoconstrictor effect of NE is not influenced by ketanserin, and 5-HT induced vasoconstriction is only to a very limited extent affected by phentolamine.

The interaction between 5-HT and other vasoactive substances such as epinephrine, NE, vasopressin, histamin, and angiotensin II has been dealt with in several studies. In intact rats for example, 5-HT doubled the blood pressure increase induced by NE (De Cree et al., 1981). Studies on blood flow in the forearm, where it was shown that the NE-induced decrease in blood flow was enhanced by virtually inactive doses of 5-HT, indicates that the amplification phenomenon also exists under in vivo conditions in man (Scroop and Walsh, 1968). The amplifying effects of 5-HT in isolated blood vessels have been studied in detail by de la Lande et al. (1966), who showed that 5-HT markedly enhances the vasoconstrictor effects of NE. We have found a potentiation also in human mesenteric arteries, whereas such an effect appears to be lacking in rat's mesenteric arteries (Moreland and Bohr, 1983).

The receptor mechanisms involved in the vasoconstrictor and potentiating effects of 5-HT have recently been investigated by Seabrook and Nolan (1983). They used isolated perfused mesenteric arteries of rat and employed different receptor antagonists. The vasoconstrictor response to 5-HT was abolished in preparations pretreated with methysergide, cyproheptadine, or fluoxetine while ketanserin antagonized the response by 90%. In contrast to this, only methysergide antagonized the amplifying effect of 5-HT, while the other three antagonists had no effect on this action. In view of the different sensitivites of the vasoconstrictor and potentiating effects of 5-HT to antagonist drugs, it was suggested that the vascular receptors mediating these actions are separate (Seabrook and Nolan, 1983). This contention is further supported by the observation that

the vasoconstrictor response to the continuous presence of 5-HT is transient, lasting less than 30 min, and shows tachyphylaxis; in contrast, the ability of 5-HT to potentiate the vasoconstrictor response to NE is still evident 90 min after the introduction of 5-HT, at a time when the receptor mechanisms mediating vasoconstriction is apparently desensitized (Seabrook and Nolan, 1983). It is also reported that the calcium entry blocker, verapamil, affects only the vasoconstrictor response to 5-HT, leaving the 5-HT induced potentiation intact (Seabrook and Nolan, 1983). This potentiating effect of pre-exposure to 5-HT was seen also when the agonist-induced contraction was slight and may therefore be different from the effect of prior agonist-induced strong contraction (Rapoport and Bevan, 1983). In the latter case the subsequent increase in responsiveness is probably due to the contraction per se rather than the agonist-receptor interaction. In both instances the effects last for at least 90 min (Rapoport and Bevan, 1983; Seabrook and Nolan, 1983), and cannot be elicited in veins as shown both in the present study and by Rapoport and Bevan (1983). Besides the assumption that the two phenomena involve different steps in the stimulus-contraction sequence of events, the detailed mechanisms involved are not known.

It was notable that also previous exposure to NE increased the contractile response to 5-HT in the mesenteric arteries. This is in contrast to findings on the rabbit ear artery where NE did not sensitize the vessel to subsequent 5-HT induced contraction; in fact, 5-HT was, in the opposite type of experiment, less efficient than histamine and KCl in sensitizing the ear artery to subsequent NE-induced contraction (Rapoport and Bevan, 1983). Since there is considerable evidence that 5-HT acts at α-adrenoceptors in the ear artery (Apperley et al., 1976; Fozard, 1976; Purdy et al., 1981), the lack of effect has been interpreted as a desensitization of the α-adrenoceptors by 5-HT, and of the 5-HT-receptors by NE, thereby masking the potentiating effects (Rapoport and Bevan, 1983). The marked mutual sensitization by NE and 5-HT presently found is thus consistent with our evidence that NE-induced contraction does not involve 5-HT receptors, and that 5-HT has little or no effect on α-adrenoceptors in the human mesenteric artery.

Acknowledgement: The authors thank Inga-Lill Bertilsson for skilful technical assistance.

REFERENCES

Apperley, E.; Humphrey, P.P.A.; Levy, G.P.: Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. Br. J. Pharmacol. 58:211-221 (1976).

Arneklo-Nobin, B.; Owman, Ch.: Adrenergic and serotonergic mechanisms in human hand arteries and veins studied by fluorescence histochemistry and in vitro pharmacology. Blood Vessels 22:1-22 (1985).

Arunlakshana, O.; Schild, H.O.: Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14:48-58 (1959).

Cocks, T.M.; Angus, J.A.: Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. Nature, Lond. 305:627-630 (1983).

Cohen, R.A.; Shepherd, J.T.; Vanhoutte, P.M.: 5-hydroxytryptamine can mediate endothelium-dependent relaxation of coronary arteries. Am. J. Physiol. 245 (Heart Circ. Physiol. 14):H403-408 (1983).

DeClerck, F.; Vanhoutte,P.: 5-Hydroxytryptamine in peripheral reactions. (Raven Press, New York 1982).

Costa, M.; Furness, J.B.; Cuello, A.C.; Verhofstad, A.A.J.; Steinbusch, H.W.J.; Elde, R.P.: Neurons with 5-hydroxytryptamine-like immunoreactivity in the enteric nervous system; their visualization and reactions to drug treatment. Neuroscience 7:351-363 (1982).

De Cree, J.; Leempoels, J.; De Cock, W.; Geukens, H.; Verhaegen, H.: The antihypertensive effects of a pure and selective serotonin-receptor blocking agent (R 41 468) in elderly patients. Angiology 32:137-144 (1981).

McCubbin, J.W.; Kaneko, Y.; Page, I.H.: Inhibition of neurogenic vasoconstriction by serotonin. Circulation Res. 11:74-83 (1962).

Dahlström, A.; Fuxe, J.: Evidence for the existence of monoamine neurons in the central nervous system. Acta Physiol. Scand. 64 Suppl. 247:1-85 (1965).

Douglas, W.W.: Histamine and 5-hydroxytryptamine (serotonin) and their antagonists; in Goodman, Goodman, Gilman, The pharmacological basis of therapeutics, pp. 609-646. (MacMillan, New York 1980).

Edvinsson, L.; Hardebo, J.E.; Owman, Ch.: Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man. Circulation Res. 42:143-151 (1978).

Edvinsson, L.; Birath, E.; Uddman, R.; Lee, T.J.-F.; Duverger, D.; Mac Kenzie, E.T.; Scatton, B.: Indoleaminergic mechanisms in brain vessels; localization, concentration, uptake and in vitro responses of 5-hydroxytryptamine. Acta Physiol. Scand. 121:291-299 (1984).

Essman, W.B.: Serotonin in health and disease, Vol 5. Spectrum, New York 1977-1978.

Fozard, J.R.: Comparative effects of four migraine prophylactic drugs on an isolated extracranial artery. Eur. J. Pharmacol. 36:127-139 (1976).

Furchgott, R.F.: The use of β-haloalkylamines in the differentiation of receptors and in the determination of dissociation constants of receptor-agonist complex. Adv. Drug Res. 3:21-55 (1966).

Furchgott, R.F.: The classification of adrenoceptors (adrenergic re ceptors). An evaluation from the standpoint of receptor theory; in Blaschko, Muscholl. Handbook of experimental pharmacology, pp. 283-335 (Springer-Verlag, Berlin, Heidelberg, New York 1972).

Furchgott, R.F.: The requirement for endothelial cells in the relaxation of arteries by acetylcholine and some other vasodilators. Tr. Pharmacol. Sci. 2:173-176 (1981).

McGrath, M.A.; Vanhoutte, P.M.: Vasodilatation caused by peripheral inhibition of adrenergic neurotransmission; in Vanhoutte, Leusen, Mechanisms of Vaso-dilatation, pp. 248-257 (Karger, Basel 1977).

Griffith, S.G.; Burnstock, G.: Immunohistochemical demonstration of serotonin in nerves supplying human cerebral and mesenteric blood-vessels. Lancet 12:561-562 (1983).

Humphrey, P.P.A.: The effects of α-adrenoceptor antagonists on contractile responses to 5-hydroxytryptamine in dog saphenous veins. Br. J. Pharmacol. 63:671-675 (1978).

Humphrey, P.P.A.: Pharmacological characterization of cardiovascular 5-hydroxytryptamine receptors; in Bevan et al, Vascular neuroeffector mechanisms, pp. 97-102 (Raven Press, New York 1983).

Mac Kenzie, E.T.; Stewart, M.; Young, A.R.; Hurray Harper, A.: Cerebro vascular actions of serotonin. Advanc. Neurology 20:77-83 (1978).

De la Lande, I.S.; Cannell, V.A.; Waterson J.G.: The interaction of serotonin and noradrenaline on the perfused artery. Br. J. Pharm. Pharmacol. 28:255-272 (1966).

Leysen, J.E.; Awouters, F.; Kennis, L.; Laduron, P.M.; Vandenberk, J.; Janssen, P.A.J.: Receptor binding profile of R 41 468 a novel antagonist of 5-HT_2 receptors. Life Sci. 28:1115-1122 (1981).

Mecca, T.E., Webb, R.C.: Serotonin and vasodilatation; in Vanhoutte, Vatner, Vasodilator mechanisms, pp. 81-90 (Karger, Basel 1984).

Moreland, R.S.; Bohr, D.F.: Prior exposure to serotonin attenuates nor epinephrine contraction in dog mesenteric artery. Blood Vessels 20:203 (1983).

Nobin, A.; Björklund, A.: Topography of the monoamine neuron systems in the human brain as revealed in fetuses. Acta Physiol. Scand. Suppl 388:1-40 (1973).

Purdy, R.E.; Hurlbut, D.E.; Rains, L.A.: Receptors for 5-hydroxytryptamine in rabbit isolated ear artery and aorta. Blood Vessels 18:16-27 (1981).

Rapoport, R.M. and Bevan, J.A.: Effect of contraction on the subsequent responsiveness and maximum contractility of the rabbit ear artery and saphenous veins in vitro. Blood Vessels 20:44-55 (1983).

Rossum, J.M. van; Brink, F.G. van den: Cumulative dose-response curves. I. Introduction to the technique. Arch. Int. Pharmacodyn. Ther. 143: 240-246 (1963).

Scroop, G.C.; Walsh, J.A.: Interactions between angiotensin, noradrenaline and serotonin on peripheral blood vessels in man. Aust. J. Exp. Biol. Med. Sci. 46:573-580 (1968).

Seabrook, J.M.; Nolan, P.L.: The vascular interaction of noradrenaline and 5-hydroxytryptamine. Eur. J. Pharmacol. 89:131-135 (1983).

Shepherd, J.T.; Vanhoutte, P.M.: The human cardiovascular system. Raven Press, New York 1979.

Steinbusch, H.W.M.: Serotonin-immunoreactive neurons and their projections in the CNS; in Björklund, Hökfelt. Handbook of chemical neuroanatomy, Vol 3. Classical transmitters and transmitter receptors in the CNS, Part II, pp. 68-125 (Elsevier, Amsterdam, New York, Oxford 1984).

Törnebrandt, K.; Nobin, A.; Owman, Ch.: Pharmacological characterization of α-adrenergic receptor subtypes mediating contraction in human mesentric arteries and veins. Blood Vessels 22:000-000 (1985).

Van Nueten, J.M.; Janssen, P.A.J.; van Beek, J.; Xhonneux, R; Verbeuren, T.J.; Vanhoutte, P.M.: Vascular effects of ketanserin (R 41468), a novel antagonist of 5-HT_2 serotonergic receptors. J. Pharmacol. Exp. Ther. 218:217-230 (1981).

Van Nueten, J.M.: Serotonergic amplification mechanisms in vascular tissues; in DeClerck, Vanhoutte. 5-Hydroxytryptamine in peripheral reactions, pp 77-82 (Raven Press, New York 1982).

Vanhoutte, P.M.: Heterogeneity of vascular smooth muscle; in: Kaley, Altura. Microcirculation, Vol. II, pp. 181-389 (University Park Press, Baltimore 1978).

Vanhoutte, P.M.; Verbeuren, T.J.; Webb, R.C.: Local modulation of adre nergic neuroeffector interaction in blood vessel wall. Physiol. Rev. 61:151-247 (1981).

Walsh, J.A.: Antagonism of methysergide of vascular effects of 5-hydroxy-tryptamine in man. Br. J. Pharmacol. 30:518-530 (1967).

VASOMOTOR EFFECTS OF NINE NEUROPEPTIDES ON ISOLATED HUMAN MESENTERIC BLOOD VESSELS

K. Törnebrandt*, A. Nobin• and Ch. Owman°

Departments of Anaesthesiology*, Surgery•, and Histology°, University of Lund, S-221 85 Lund, Sweden

Running title: Neuropeptides and isolated human mesenteric vessels

Key words: Neuropeptide Y (NPY); substance P (SP); calcitonin gene-related peptide (CGRP); vasoactive intestinal polypeptide (VIP); vascular smooth muscle; substance P (SP)-antagonist

Summary

The neuropeptides, neuropeptide Y (NPY), substance P (SP), vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), neurotensin, lysyl-bradykinin (bradykinin), somatostatin, met- and leu-enkephalin were tested for their smooth muscle activity in isolated human mesenteric arteries and veins. Only NPY regularly contracted both arteries and veins. The intrinsic activity was about 10 times higher than that earlier found for norepinephrine and 5-hydroxytryptamine (5-HT), while the potencies were in the same range. NPY probably acted directly on smooth muscle receptors, since tetrodotoxin does not affect the contraction. Alpha-adrenergic and 5-HT_2 antagonists did not affect the response either. Somatostatin contracted the veins, but not the arteries, in an inconsistent but concentration-dependent way. On the other hand neurotensin caused a weak, irregular contraction in the arteries. - The other neuropeptides were without contractile effect.

In vessels precontracted with prostaglandin $F_{2\alpha}$ or uridine triposphate CGRP, bradykinin, and SP regularly dilated both arteries and veins in a concentration-dependent way. CGRP seemed to be the most potent dilator. VIP and somatostatin regularly caused a moderate dilatation in the arteries, whereas in the veins, somatostatin caused no dilatation and VIP-induced dilatation was irregular. In both types of vessels met-enkephalin seldom gave any significant dilatation and no dilatation occurred in the presence of leu-enkephalin, NPY, or neurotensin. - The SP-antagonist ($D\text{-Arg}^1$, $D\text{-Trp}^{7,9}$, Leu^{11})-SP (Spantide) interacted with the SP-induced dilatation in a not purely competetive way.

Introduction

Immunocytochemical investigations have revealed that a variety of neuropeptides are localized in perivascular nerve fibres. Such peptide-containing vascular nerves seem to be present in all mammalian species, including man, to a varying extent in different vascular beds. Thus, nerves containing substance P (SP), vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), and peptide histidine isoleucine (PHI) are found around cerebral vessels (1, 2) and in association with peripheral vessels supplying exocrine glands, lungs, heart, gut and the urogenital tract (3, 4, 5).

Among the peptides mentioned, SP has the longest history (6). The peptide probably mediates the local vasodilatation induced during antidromic stimulation of sensory nerves (7, 8). On the other hand, in high doses, it acts as vasoconstrictor of rabbit's mesenteric and rat's portal veins (9). VIP is also a strong vasodilator (3, 9, 10) which appears to coexist with PHI in cholinergic nerve fibres, where it is a strong candidate as mediator of the atropine- resistent vasodilatation upon parasympathetic nerve stimulation (11, 12). In a corresponding manner, NPY seems to be present in sympathetic fibres together with norepinephrine (5, 13). Recent evidence indicates that these two transmitters cooperate in vascular reactions both at a prejunctional and postjunctional level (14, 15, 16, 17). Also enkephalin interferes with adrenergic neurotransmission and depresses vascular responses to sympathetic nerve stimulation (18).

Application of recombinant DNA and molecular biological techniques has revealed the existence of previously unknown neuropeptides. Calcitonin gene-related peptide (CGRP) has been localized in sensory neurons (19) and has been shown to have strong vasodilatory actions (20). It is present in perivascular nerves of the brain and peripherial organs of laboratory animals and man, and it exerts its vasodilatory effect both through direct influence on the vascular smooth musculature and by inhibition of neurogenic vasoconstriction (20, 21, 22).

Peptidergic control of the vascular smooth muscle may thus be an important general phenomenon parallel to or together with the established transmitters in the autonomic system. In the present study a large series of neuropeptides were tested for their postjunctional smooth muscle activity in human mesenteric arteries and veins.

Material and Methods

Material

Small segments of mesenteric arteries and veins were obtained from approximately 100 men and women, aged 20 - 70 years, undergoing surgery due to non-endocrine ventricular or intestinal malignancy. Morphine chloride and scopolamine bromide, or droperidol and fentanyl, were given as preanesthetic medication. Anesthesia was induced by pentothal sodium, or droperidol together with fentanyl administered intravenously. Intubation was facilitated by succinylcholine and the patients were ventilated with dinitrous oxide and oxygen. Supplementary doses of pancuronium bromide and fentanyl were given as needed. The patients were informed about the purpose of the investigation and had given their consent to the excision of the vascular preparations.

Vascular preparations

Arteries (diameter 0.4-0.9 mm) and veins (diameter 0.5-1.7 mm) were dissected from terminal branches of the mesenteric vessels close to the intestines and immediately transferred to a cold modified Krebs-Ringer solution (for composition, see below). They were kept at +4 ^{o}C in a refrigerator until the experiments were performed on the same or the next day. A 5-mm long piece was mounted between two L-formed metal holders in a 5-ml manteled organ bath for recording of circular motor activity. The organ bath contained a modified Krebs-Ringer solution of the following composition (mM concentration): NaCl 118; KCl 4.5; $CaCl_2$ x 2 H_2O 2.5; $MgSO_4$ x 7 H_2O 1.0; $NaHCO_3$ 25; KH_2PO_4 1.0; glucose 6.0. The temperature in the bath was thermostatically maintained at +37 ^{o}C and the buffer solution was continuously aerated with a mixture of 95 % O_2 and 5 % CO_2 giving pH 7.40 (range 7.35-7.45). The contractile and dilator responses were recorded isometrically with Grass FT03C force-displacement transducers and monitored on a Grass 79D polygraph. An initial load of 20-35 mN (23) was applied to the vessels, followed by a 60-90 min accommodation period before the tests with agonists and antagonists were started.

In vitro recordings

The peptides were added to the organ bath in a cumulative way (24) in order to obtain full concentration-response curves. When contractile activity was tested, 10^{-6} M propranolol was present in the bath in order to block any β-receptor mediated dilatory effects (cf. 25). When dilatory responses were studied, the vessels were given an active tone by 10^{-7} to 10^{-6} M of either prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or uridine triphosphate (UTP). This tonic contraction was found to remain at a steady level for at least 30 min. During that time varying concentrations of neuropeptides were added.

Curves were plotted either in absolute values or in terms of relative vasomotor response. The semilogarithmic concentration-response curves were used to estimate maximum response (E_{Am}) and concentration of agonists giving half maximum response (EC_{50}). Student's t-test was applied to compare differences between mean values.

Drugs

The following compunds were tested: L-arterenol hydrochloride, 5-hydroxytryptamine (5-HT) creatinine sulphate (Sigma), carbamylcholine chloride (Aldrich), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Amoglandin, Recip), uridine triphosphate (UTP; Sigma), phentolamine (Regitin, Ciba-Geigy), ketanserin tartrate (Janssen), propranolol hydrochloride (Inderal, ICI), substance P (Sigma), lysyl-bradykinin (Kallidin), leu-enkephalin, met-enkephalin, somatostatin 14, vasoactive intestinal polypeptide (VIP) (all from CRB), neuropeptide Y (NPY), neurotensin, calcitonin gene-related peptide (CGRP) (all from Peninsula), atropine sulphate (Alcon), and ($D\text{-}Arg^{1}$, $D\text{-}Trp^{7,9}$, Leu^{11})-SP (Spantide, Bachem).

Results

Vasoconstriction

Cumulative administration of NPY resulted in concentration-dependent contraction of both mesenteric arteries and veins with EC_{50} values of (3.0 $\pm$ 0.1) x 10^{-8} M and (1.8 $\pm$ 0.6) x 10^{-8} M, respectively (Table I; Fig. 1.). The general characteristics of the contractile response was similar in vessels from a number of different patients. However, both the potency (as judged from the E_{Am} values) and the intrinsic activity (EC_{50}) varied considerably between different vascular preparations and sometimes also from the same patient. The NPY contractions were not modified by the presence of the alpha-adrenoceptor antagonist, phentolamine (10^{-7} M), or the 5-hydroxytryptamine (5-HT) antagonist, ketanserin (10^{-7} M).

Administration of neurotensin elicited a weak contraction in one of 13 arterial preparations from five patients (Table I). No contractile response was registered in mesenteric veins after neurotensin application. In arteries somatostatin was without contractile effect (11 preparations, four patients), but in veins the peptide induced contraction (four of 10 preparations) in an inconsistent but concentration-dependent way.

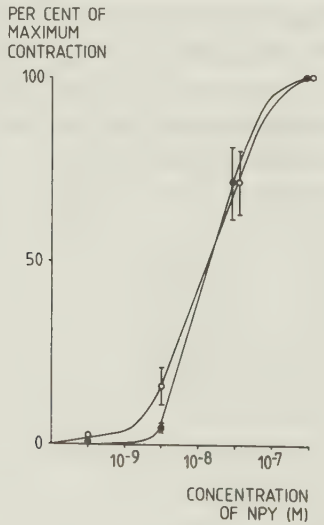


Fig. 1. Contractile response to NPY in mesenteric arteries (●) and veins (o). The 100 per cent level represents 9.2 mN in the arteries and 7.0 mN in the veins. The values are means $\pm$ SEM of 6 determinations in each group.

Table I. Contractile effect of various neuropeptides in human mesenteric arteries and veins.

	MESENTERIC ARTERIES				MESENTERIC VEINS			
Peptide	No of vessels tested	No of vessels contracting	EC_{50}, M (mean±SEM)	E_{Am}, mN (mean±SEM)	No of vessels tested	No of vessels contracting	EC_{50}, M (mean±SEM)	E_{Am}, mN (mean±SEM)
Lysyl-bradykinin	13	0			8	0		
Substance P	8	0			10	0		
Leu-enkephalin	11	0			6	0		
Met-enkephalin	11	0			9	0		
Somatostatin	11	0			10	4		5.0 ± 2.3
VIP	10	0			11	0		
NPY	21	21	$(3.0\pm1.0)\times10^{-8}$ (n=6)	9.2 ± 1.8	14	14	$(1.8\pm0\text{-}6)\times10^{-8}$ (n=7)	7 ± 1.3
Neurotensin	13	1		1.4	7	0		
CGRP	11	0			8	0		

SP, VIP, met- and leu-enkephalin, CGRP, and bradykinin were also tested for their contractile potency in the mesenteric vessels. None of them showed any concentration-dependent constriction in doses up to 10^{-4} M (Table I).

Vasodilatation

The dilatory response was tested in vessels activily contracted with $PGF_{2\alpha}$ or UTP (summarized in Table II). Cumulative application of bradykinin, SP, somatostatin, VIP, and CGRP regulary resulted in a concentration-dependent dilatation of the mesenteric arteries (Fig. 2a). The intrinsic dilatory activity of these five peptides (comparison of the EC_{50} values) was in the order somatostatin $\geq$ SP = VIP $\geq$ CGRP $\geq$ bradykinin. The intrinsic activity of somatostatin was, however, difficult to calculate due to a non-sigmoid shape of the concentration-response curves. As evidenced from the E_{Am} values, CGRP and bradykinin were the most potent dilators in the arteries, followed in order by SP, VIP and somatostatin.

In the veins, vasodilation was regularly elicited by bradykinin, SP, and CGRP with E_{Am} values similar to one another (Table II; Fig. 2b). The rank of intrinsic activity resembled that in the arteries (SP > CGRP > bradykinin). VIP dilated nine of 15 veins with an intrinsic activity similar to that for CGRP, and a potency as for bradykinin, SP, and CGRP.

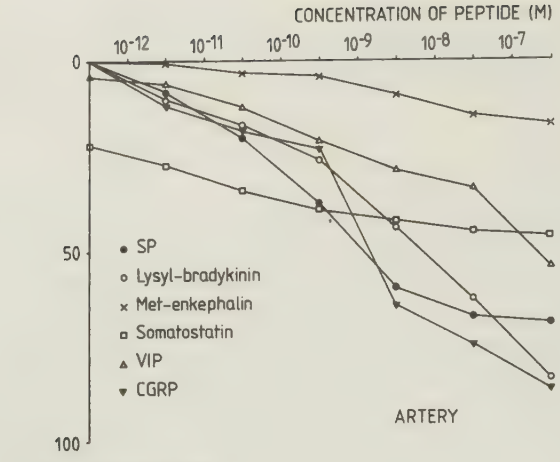
In both arteries and veins met-enkephalin caused a slight dilatation in a minority of the vessels tested (Table II; Fig. 2a and b). Leu-enkephalin, neurotensin, and NPY did not induce any dilatory responses in either arteries or veins. In addition, the veins did not dilate with somatostatin.

The dilatory effect of SP was inhibited in both mesenteric arteries and veins by (D-Arg^1, D-$Trp^{7,9}$, Leu^{11})-SP (Spantide), which is a SP antagonist. In the arteries there was a dextral shift of the concentration-response curves to SP with increasing doses of the antagonist. The antagonist also induced a reduction in the maximum dilatory response (Fig. 3a). A comparable dextral shift in the concentration-response curves was obtained in the veins exposed to increasing doses of the antagonist whereas no effect on the maximum response was registered (Fig. 3b). The shift in the concentration-response curves from the individual experiments was, however, varying and somewhat inconsistent which made it difficult to obtain Schild plots. The SP analogue (D-Arg^1, D-$Trp^{7,9}$, Leu^{11})-SP, had no contractile or dilatory effect of its own in the concentrations tested (10^{-9} to 10^{-6} M).

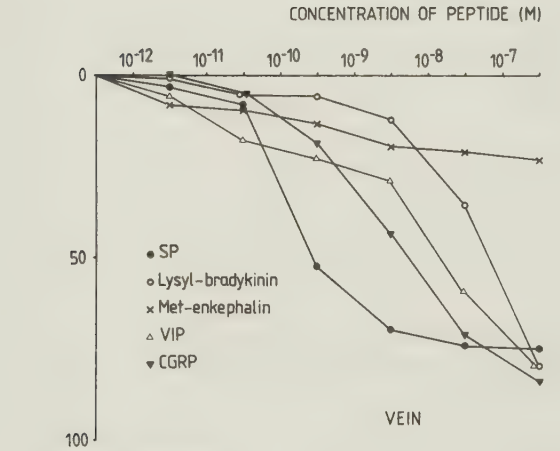
Table II. Dilatory effect of various neuropeptides in human mesenteric arteries and veins.

Peptide	MESENTERIC ARTERY		MESENTERIC VEIN	
	Per cent dilatation of PG-induced contraction (n)	EC_{50}, M (n)	Per cent dilatation of PG-induced contraction (n)	EC_{50}, M (n)
Lysyl-bradykinin	83 ± 10 (8)	$(9.5\pm5.3)\times10^{-9}$ (8)	79 ± 6 (7)	$(3.8\pm1.4)\times10^{-8}$ (6)
Substance P	68 ± 5 (20)	$(3.0\pm0.7)\times10^{-10}$ (20)	73 ± 13 (9)	$(2.8\pm1.2)\times10^{-10}$ (6)
Leu-enkephalin	– (27)	–	– (15)	–
Met-enkephalin	– (21); 16 (2)	– (21); 1×10^{-9} (2)	– 13; 23 (2)	– 13; 1×10^{-9} (2)
Somatostatin	46 ± 8 (6)	1×10^{-11} (5)	– (8)	–
VIP	53 ± 11 (12)	$(6.1\pm3.2)\times10^{-10}$ (4)	– (6); 78±10 (9)	– (6); $(4.4\pm1.8)\times10^{-9}$ (5)
NPY	– (6)	–	– (8); 10±3 (3)	–
Neurotensin	– (12)	–	– (6)	–
CGRP	86 ± 8 (6)	1×10^{-9} (2)	83 ± 7 (7)	$(6.4\pm3)\times10^{-9}$ (6)

Prostaglandin $F_{2\alpha}$ (PG). EC_{50} = agonist concentration producing half maximum contraction; E_{Am} = maximum contraction; K_B = dissociation constant for receptor-antagonist complex; pA_2 = intercept of regression line with abscissa in Schild plot. Significance levels (Student's t-test): * $0.05 > p > 0.01$; ** $0.01 > p > 0.001$; *** $p < 0.001$.

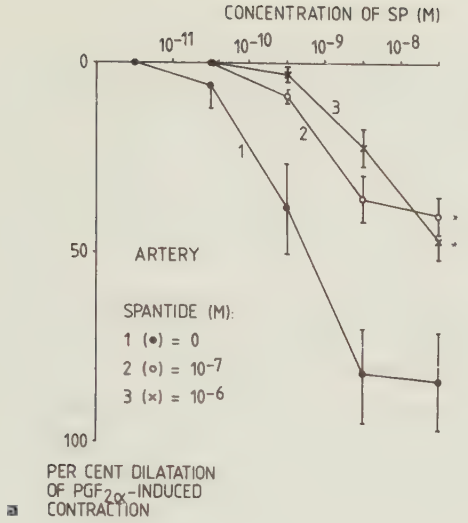


a

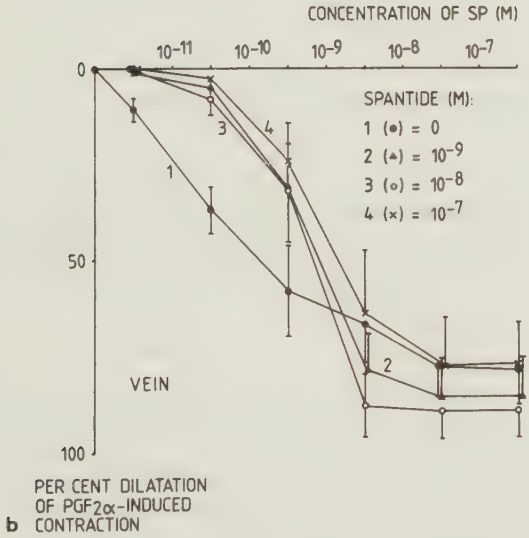


b

Fig. 2. a Dilatory response to SP (●; n = 20), lysyl-bradykinin (o ; n = 8), met-enkephalin (x ; n = 2), somatostatin (□ ; n = 6), VIP (Δ; n = 12), and calcitonin gene-related peptide (CGRP) (▼ ; n = 6) in mesenteric arteries precontracted with $PGF_{2\alpha}$ 10^{-6} M.
b Dilatory response to SP (●; n = 9), lysyl-bradykinin (o; n = 7), met-enkephalin (x; n = 2), VIP (Δ ; n = 5), and CGRP (▼ ; n = 7) in mesenteric veins precontracted with $PGF_{2\alpha}$ 10^{-6} M.



a



b

Fig. 3. Interaction between the SP-antagonist (D-Arg[1], D-Trp[7,9], Leu 11), Spantide, and SP in precontracted (10^{-6} M $PGF_{2\alpha}$) mesenteric arteries a and veins b. Values are means and SEM of 4-7 arteries and 4-9 veins. Difference from values without antagonist: *0.05 > p > 0.01.

Discussion

Among the neuropeptides (SP, VIP, NPY, CGRP, neurotensin, bradykinin, somatostatin, and met- and leu-enkephalin) presently tested on human mesenteric arteries and veins, only NPY induced consistent and potent vasoconstriction. The intrinsic activity of NPY on these vessels was about 10 times higher than that found for norepinephrine and 5-HT (23, 26), whereas the potencies of these three substances were in the same range. Tetrodotoxin does not affect the responses to NPY (27) suggesting that the peptide acted directly on smooth muscle receptors. Our results are in agreement with the vasocontrictory and hypertensive effect of NPY following intravenous administration (13). NPY appears to exhibit only a weak vasoconstriction in peripheral arteries of rabbits (14). The effect of NPY was not mediated by alpha-adrenergic or 5-HT_2 receptors as evidenced from the lack of inhibition with phentolamine and ketanserin. Also neurotensin constricted the arteries, though not regularly. A similar contractile effect induced by neurotensin on smooth muscle cells has been reported in isolated taenia coli (28). Immunocytochemistry has so far failed to demonstrate its presence in peripheral vascular nerves, whereas the peptide is located in endocrine cells and nerves of the distal ileum (27, 28).

The peptides tested more unanimously induced a dilatory response in the mesenteric vessels. The effect was generally less common and less pronunced in the veins. This was probably not due to a lower inherent ability of the veins to dilate, because SP constantly induced the same relative response in both types of vessels.

The dilatory action of SP appeared to be effectuated through specific receptors as evidenced by the inhibition with (D-Arg^1, D-$Trp^{7,9}$, Leu^{11})-SP (Spantide) (30). These receptors are conceivably located on the endothelial cells, which mediate the SP induced dilatation (31). A detailed pharmacological analysis of the receptor involved could not be performed, since the interaction between SP and (D-Arg^1, D-$Trp^{7,9}$, Leu^{11})-SP (Spantide) was not purely competitive. This may be explained by the histamine-releasing properties of the antagonist (32).

Besides SP, also CGRP produced a significant degree of dilatation in the human mesenteric vessels. The potent dilatory effect of CGRP on human blood vessels is in accordance with previous effects in vitro (21) and with results obtained after intradermal injections of the substance (20). However, when injected into the brain ventricles, CGRP produced a significant raise in mean arterial blood pressure probably caused by central stimulation of the noradrenergic sympathetic outflow (22).

The results with bradykinin indicates that vasodilatation induced by this peptide comprises one component in the vascular reactions of patients with carcinoid tumours. These are known to be rich in bradykinin activiting enzymes (33, 34). It has been suggested that bradykinin acts by releasing a neurogenic mediator, possibly SP (35). This assumption is further supported by the blocking effects of SP-antagonists on bradykinin actions (36).

It was notable that VIP, generally considered as a strong vasodilator, was without consistent dilatory effect on the mesenteric veins. This may correspond to the scarcity of VIP-ergic innervation of peripheral veins (37).

In conlusion, a series of neuropeptides, the majority of which have been demonstrated in perivascular nerve fibres, exert significant motor effects in human blood vessels. Among the neuropeptides presently tested, NPY appears to be the most potent contractor of human mesenteric blood vessels, and CGRP seems to be the most potent dilator.

Acknowledgement

The authors thank Inga-Lill Bertilsson for skilful technical assistance. The study was supported by grants from the Swedish Medical Research Council (grants No. 12X-712 and 14X-732) and from Janssen Leo Pharma AB.

REFERENCES

1. Hardebo, J-E., Andersson, J., Hanko, J. and Owman, Ch., Purinergic and peptidergic vasomotor mechanisms in the brain, In P.M. Vanhoutte, S.F. Vatner (Eds.), Vasodilator Mechanisms, 3rd International Symposium on Mechanisms of Vasodilatation, Sidney, August 25-27, 1983, Karger, Basel, 1984, pp. 128-137.

2. Owman, Ch., Andersson, J., Hanko, J. and Hardebo, J-E., Neurotransmitter amines and peptides in the cerebrovascular bed. In E.T. MacKenzie, J. Seylaz and A. Bēs (Eds.), L.E.R.S. Monograph Series, vol. 2: Neurotransmitters and the cerebral circulation, Raven Press, New York, 1984, pp. 11-38.

3. Uddman, R., Alumets, J., Edvinsson, L., Håkanson, R. and Sundler, F., VIP nerve fibres around peripheral blood vessels. Acta Physiol. Scand., 112 (1981) 65-70.

4. Lundberg, J.M., Hökfelt, T., Änggård, A., Lundblad, L., Saria, A., Fahrenkrug, J. and Terenius, L., Neuropeptides with vascular activity: VIP, PHI, NPY and substance P, In P.M. Vanhoutte, S.F. Vatner (eds.), Vasodilator Mechanisms, 3rd International Symposium on Mechanisms of Vasodilatation, Sidney, August 25-27, 1983, Karger, Basel, 1984, pp. 60-69.

5. Uddman, R., Ekblad, E., Edvinsson, L., Håkanson, R. and Sundler, F., Neuropeptide Y-like immunoreactivity in perivascular nerve fibres of the guinea-pig. Regul. Peptides, 10 (1985) 243-257.

6. Euler, U.S. von and Gaddum, J.H., An unidentified depressor substance in certain tissue extracts, J. Physiol., Lond., 72 (1931) 74-87.

7. Lembeck, F. and Holzer, P., Substance P as a neurogenic mediator of antidromic of vasodilation and neurogenic plasma extravasation, Naunyn Schmiedebergs Arch. Pharmacol., 310 (1979) 175-183.

8. Hökfelt, T., Vincent, S., Dalsgaard, C-J., Skirboll, L., Johansson, O., Schultzberg, M., Lundberg, J.M., Rosell, S., Pernow, B. and Jancso, G., Distribution of substance P in brain and periphery and its possible role as a co-transmitter, Ciba Foundation Symposium 91, Pitman, London, 1982, pp. 84-106.

9. Hellstrand, P. and Järhult, J., Effects of nine different gastrointestinal polypeptides on vascular smooth muscles in vitro, Acta Physiol. Scand., 110 (1980) 89-94.

10. Larsson, L., Edvinsson, L., Fahrenkrug, J., Håkanson, R., Owman, Ch., Schaffalitzky de Muckadell, O. and Sundler, F., Immunohistochemical localization of a vasodilatory polypeptide (VIP) in cerebrovascular nerves, Brain Res. 113 (1976) 400-404.

11. Lundberg, J.M., Änggård, A., Fahrenkrug, J., Hökfelt, T. and Mutt, V., Vasoactive intestinal polypeptide (VIP) in cholinergic neurons of exocrine glands. Possible functional significance of co-existing transmitters for vasodilatation and secretion, Proc. Natl. Acad. Sci. USA, 77 (1980) 1651-1655.

12. Lundberg, J.M., Fahrenkrug, J., Hökfelt, T., Martling, C-R. Larsson, O., Tatemoto, K. and Änggård, A., Co-existense of peptide HI (PHI) and VIP in nerves regulating blood flow and bronchial smooth muscle tone in various mammals including man, Peptides, 5 (1984) 593-606.

13. Lundberg, J.M. and Tatemoto, K., Pancreatic polypeptide family (APP, BPP, NPY and PYY) in relation to sympathetic vasoconstriction resistant to α-adrenoceptor blockade, Acta Physiol. Scand., 116 (1982) 393-402.

14. Edvinsson, L., Ekblad, E., Håkanson, R. and Wahlestedt, C., Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels, Br. J. Pharmacol., 83 (1984a) 519-525.

15. Edvinsson, L., Emson, P., McCulloch, J., Tatemoto, K. and Uddman, R., Neuropeptide Y: Immunocytochemical localization to and effect upon feline pial arteries and veins in vitro and in situ, Acta Physiol. Scand., 122 (1984b) 155-163.

16. Ekblad, E., Edvinsson, L., Wahlestedt, C., Uddman, R., Håkanson, R. and Sundler, F., Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibres, Regulatory Peptides, 8 (1984) 225-235.

17. Hanko, J., Törnebrandt, K., Hardebo, J.E., Kåhrström, J., Nobin, A. and Owman, Ch., Neuropeptide Y induces and modulates vasoconstriction in intracranial and peripheral vessels of animals and man, Circ. Res., (1985a) Submitted for publication.

18. Knoll, J., Neuronal peptide (enkephalin) receptors in the ear artery of the rabbit, Eur. J. Pharmacol., 39 (1976) 403-507.

19. Rosenfeld, M.G., Mermod, J.J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., Rivier, J., Vale, W.W. and Evans, R.M., Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing, Nature, 304 (1983) 129-135.

20. Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R. and MacIntyre, Calcitonin gene-related peptide is a potent vasodilator, Nature, 313 (1985) 54-56.

21. Hanko, J., Hardebo, J-E., Kåhrström, J., Owman, Ch. and Sundler, F., Calcitonin gene-related peptide (CGRP) is present in cerebrovascular nerve fibres and dilatates pial peripheral arteries, Neuroscience Letters, (1985b) In press.

22. Fisher, L., Kikkawa, D., Rivier, J., Amara, S., Evans, R., Rosenfeld, M., Vale, W. and Brown, M., Stimulation of noradrenergic sympathetic outflow by calcitonin gene-related peptide, Nature, 305 (1983) 534-535.

23. Törnebrandt, K., Nobin, A. and Owman, Ch., Pharmacological characterization of alpha-adrenergic receptor subtypes mediating contraction in human mesenteric arteries and veins, Blood vessels, 22 (1985a) 000-000.

24. Rossum, J.M. van and Brink, F.G. van den, Cumulative dose-response curves. I. Introduction to the technique, Archs Int. Pharmacodyn. Thér., 143 (1963) 240-246.

25. Edvinsson, L., Hardebo, J.E. and Owman, Ch., Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man, Circ. Res., 42 (1978) 143-151.

26. Törnebrandt, K., Nobin, A. and Owman, Ch., Serotonergic smooth muscle receptors in human mesenteric arteries and veins. Blood vessels, (1985b) submitted for publication.

27. Hanko. J., Törnebrandt, K., Hardebo, J.E., Kåhrström, J., Nobin, A. and Owman, Ch., Neuropeptide Y induces and modulates vasoconstriction in intracranial and peripheral vessels of animals and man. Regulatory Peptides, (1985) submitted.

28. Leander, S., Ekman, R., Uddman, R., Sundler, F. and Håkanson, R., Neuronal cholecystokinin, gastrin-releasing peptide, neurotensin, and β-endorphin in the intestine of the guinea-pig. Distribution and possible motor functions. Cell Tissue Res., 235 (1984) 521-531.

29. Sjölund, K., Sandén, G., Håkanson, R. and Sundler, F., Endocrine cells in human intestine: an immohistochemical study, Gastroenterology, 85 (1983) 1120-1130.

30. Rosell, S., Björkroth, U. and Folkers, K., Subpopulations of substance P (SP) receptors and separate eledoisin receptors. In Substance P Dublin 1983. Eds. P. Skrabanek and D. Powell. Boole Press, Dublin 1983, pp. 61-62.

31. Furchgott, R., The requirement for endothelial cells in the relaxation arteries by acetylcholine and some other vasodilators, Trends pharmacol Sci., 2 (1981) 173-176.

32. Håkanson, R., Hörig, J. and Leander, S., The mechanism of action of a substance P antagonist (D-Pro^2, D-Trp^{7,9})-SP, Br. J. Pharmacol., 77 (1982) 697-700.

33. Oates, J.A., Melmon, K., Sjoerdsma, A., Gillespie, L. and Mason, D.T., Release of a kinin peptide in the carcinoid syndrome, Lancet, 1 (1964) 514-517.

34. Smith, A.N. and Zeitlin, I.J., The role of bradykinin in vasomotor aspects of the carcinoid and dumping syndromes, Br. J. Surg, 53 (1966) 867-869.

35. Butler, J.M., Cole, D.F. and Zhang, S.W., Effect of trigeminal denervation on in vitro responses of rabbit iris sphincter muscle to bradykinin and substance P, J. Physiol., (London), 316 (1981) 18.

36. Bynke, G., Håkanson, R., Hörig, J. and Lenader, S., Bradykinin contracts the pupillary sphincter and evokes ocular inflammation through release of neuronal substance P, Eur. J. Pharmacol., 91 (1983) 469-475.

Neuropeptide Y Induces and Modulates Vasoconstriction in Intracranial and Peripheral Vessels of Animals and Man

John H. Hanko[1], Kenneth Törnebrandt[2], Jan Erik Hardebo[1], Jan Kåhrström[3], Anders Nobin[4] and Christer Owman[3]

From the Departments of Histology[3], Neurology[1], Anesthesiology[2] and Surgery[4], University of Lund, S-223 62 Lund, Sweden

Abbreviated title: NPY induces and modulates vasoconstriction

Acknowledgement of support: Supported by grants from the Swedish Medical Research Council (No. 14X-732 and 12X-712).

Address for correspondence: Prof. Christer Owman, PhD, MD
Department of Histology, University of Lund
Biskopsgatan 5
S- 223 62 LUND
Sweden (Phone: 46-46-120799)

SUMMARY. Neuropeptide Y (NPY) has recently been reported to coexist with norepinephrine (NE) in central as well as peripheral adrenergic nerves. NPY-containing nerve fibres are particularly numerous around blood vessels. Studies were performed on isolated pial arteries as well as on arteries and veins from several peripheral vascular beds from rabbit, cat and man. NPY induced a varying degree of direct contraction of the vessels with an E_{Am} up to 15 mN. Pial arteries were more sensitive than peripheral arteries to NPY (mean EC_{50} = 7.6 x 10^{-9} M). The presence of NPY did not cause any consistent or significant potentiation of the contractile response to NE in any of the vessels tested. Transmural electrical stimulation of the perivascular nerves (including blockade with tetrodotoxin) was performed with mainly rabbits auricular artery. Blocking experiments confirmed that the neurogenic contraction was mediated by adrenergic-type fibres. NPY caused a concentration-related potentiation of the neurally evoked contractile response. In some experiments it was observed that NPY also potentiated tetrodotoxin-resistant contractions obtained during electrical field stimulation. It is concluded that NPY potentiates the effect of NE during sympathetic nerve activation primarily through a presynaptic effect.

Index terms: Isolated peripheral vessels, isolated cerebral vessels, contraction in vitro, norepinephrine, neuropeptide Y, transmural nerve stimulation, neuromodulation.

Introduction

A population of central and peripheral noradrenergic neurons contain neuropeptide Y (NPY), a 36 amino acid peptide recently isolated from porcine brain (Tatemoto et al., 1982; Lundberg et al., 1982, 1983; Adrian et al., 1983; Hökfelt et al., 1983; Everitt et al., 1984). The amino acid sequence displays a considerable degree of homology (Tatemoto et al., 1982) with both bovine pancreatic polypeptide (17 amino acids in common), avian pancreatic polypeptide (20 amino acids in common) and polypeptide YY (25 amino acids in common). This similarity probably explains early reports on the presence of avian pancreatic polypeptide in adrenergic neurons (Lundberg et al., 1980; Hunt et al., 1982; Uddman et al., 1982). There is thus reason to believe that the immunoreactive substance with a neural localization demonstrated in these studies rather is identical with NPY.

NPY-immunoreactive nerve fibres are particularly numerous in the walls of blood vessels (Furness et al., 1983; Stjernquist et al., 1983; Sundler et al., 1983; Terenghi et al., 1983; Uddman et al., 1984). Cerebral arteries are equipped with NPY-containing nerve endings which probably originate from cell bodies in the superior cervical sympathetic ganglia (Uddman et al., 1982; Edvinsson et al. 1983, 1984b; Ekblad et al., 1984; Hardebo et al., 1984; Owman et al., 1984). The primary action of NPY in smooth muscle tissues seems to be a direct contractile response, but in view of the coexistence between NPY and norepinephrine (NE) in sympathetic nerves it has been suggested that NPY also has a prejunctional adrenergic effect (Lundberg et al., 1982).

The present report extends studies on direct vascular effects of NPY also to human blood vessels in comparison with central and peripheral blood vessels of rabbit and cat. It analyses quantitatively the possible prejunctional as well as postjunctional interaction between NPY and NE in the different types of arteries and veins.

Material and Methods

Material

Human pial arteries (300-600 μm in diameter) overlying macroscopically intact tissue were obtained during temporal lobe resection in conjunction with brain tumor operations in 3 patients. Mesenteric arteries and veins were received from 20 patients undergoing intestinal resection. The patients were informed in detail about the purpose of the investigation

and had given their consent to the exicision of the vascular preparations. Six cats and 30 rabbits were killed by exsanguination under pentobarbitone anesthesia. Middle cerebral arteries and mesenteric arteries and veins were removed from the cats. The basilar, mesenteric, and central ear arteries as well as mesenteric veins were taken from the rabbits. All preparations were immediately placed in an ice-cold Krebs-Ringer solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2x2H_2O$ 1.0, $MgSO_4x7H_2O$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2, glucose 5.6. The vessels were carefully dissected free from connective tissue. Most of them were used for testing on same day, but some were stored in the buffer solution in a refrigerator at +4 °C overnight.

Five-mm long circular segments of the vessels were mounted between two L-shaped metal holders in 2.5-ml baths containing Krebs-Ringer solution (Hardebo et al., 1985). Bath temperature was maintained thermostatically at 37 °C and the pH was kept steady around 7.40 (range 7.35-7.45) by continuous bubbling with a gas mixture consisting of 88.5% O_2 and 11.5% CO_2. The vascular tension was recorded isometrically with a Grass FT03C force-displacement transducer and registered on a Grass model 79D polygraph. The vessels were given an initial passive load of 5 mN (pial arteries) or 10-20 mN (peripheral vessels) and allowed to equilibrate for 90 min before the experiments were started. The load applied was of a magnitude that would give a maximum contractile response in each particular type of vessel, as tested in separate experiments (Edvinsson et al., 1974; Törnebrandt et al., 1985). The vessel tension decreased by about 60% during the accomodation period.

NPY and other substances were dissolved in 0.9% saline or Krebs-Ringer solution and administered into the baths in a total volume of approximately 100 µl. In the case of NE, ascorbic acid (0.1 mM) and $Na_2CaEDTA$ (0.029 mM) were added to the solution in order to reduce amine oxidation. Two cumulative concentration-response (cr) curves were run with NE after which NPY was introduced into the bath, and a new cr curve for the amine was obained after 10 min. Finally, another curve after wash-out of NPY was registered. Several segments of each vessel from individual animals and patients were mounted, and several identical tests were thus often run on the same vessel.

Electrical field stimulation

Platinum electrodes (0.3 mm in diameter and 4 mm apart) were mounted on both sides of the vessels (Hardebo et al., 1985). Extensive studies have clearly shown that it is not possible to obtain a purely neurogenic vasoconstriction when pial vessels from the species presently tested are submitted to electrical field stimulation (Hardebo et al., 1985). Consequently, only peripheral vessels were utilized to test whether NPY was able to modulate electrically induced vasoconstrion, namely central ear arteries from rabbits and mesenteric arteries and veins from man, cat and rabbit. Electrical stimulation was given as a 10-sec train of square wave pulses every 6 min using a Grass S44 stimulator with an additional external power supply of 45 V. Voltage was continuously measured on an oscilloscope. A stimulation current of 9 V, measured over the electrodes, with a duration of 0.1 msec and a frequency of 8 Hz resulted in a vasoconstriction that could be totally blocked by 5×10^{-7} M tetrodotoxin (TTX) and therefore was considered to be of neurogenic origin. If the current was increased over 10 V or if the stimulus duration was extended to 0.15 msec at 9 V and 8 Hz the constriction could no longer be completely blocked by TTX. Some stimulation experiments were run with stimulation parameters of 10 V, 0.3 msec and 8 Hz, which induce a vasoconstriction that is not purely neurogenic.

Compounds used

Atropine sulphate (Alcon), neuropeptide Y (Peninsula), L-arterenol hydrochloride (Sigma), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; Amoglandin, Kabi-Vitrum), phentolamine methanesulphonate and gunanethidine (Ciba-Geigy), rauwolscine hydrochloride (Roth) and tetrodotoxin (Sanyo). The concentrations below are given as final molar concentration in the bath.

Analysis of data

The full cr curves were illustrated as per cent of maximum contractile response. Differences in agonistic effects were evaluated on the basis of EC_{50} values (concentration of agonist giving half maximum response) or pD_2 values (negative logarithm of the EC_{50} value). Statstical analysis was carried out with Student´s t-test.

Results

Direct smooth muscle effects of NPY

The direct vasomotor effect of NPY in arteries as well as veins was a contractile response, whereas no dilatation was seen as tested on vessels given a previous contraction with 10^{-6}M $PGF_{2\alpha}$. The NPY-induced contraction developed gradually up to a plateau, which remained until wash-out, and it was concentration-dependent (Fig. 1).

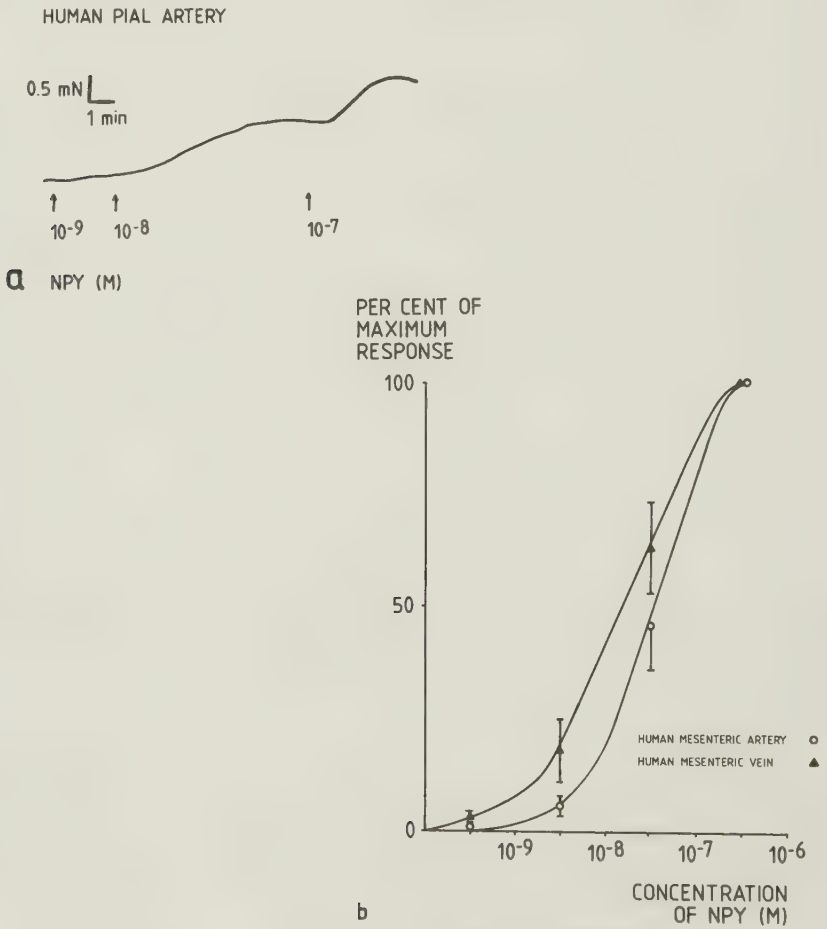


Fig. 1. NPY-induced concentration-dependent vasoconstriction in (a), human pial artery from the temporal lobe (b), human mesenteric artery (o), and vein (▲).

However, there was a considerable variability in that almost one third of the vessels did not respond at all to NPY (doses up to 10^{-6} M tested) under conditions when an expected degree of contraction was seen with NE or $PGF_{2\alpha}$. The amount of contraction was also subject to variation, but often reached approximately 15 mN in both arteries and veins at the highest NPY doses tested (10^{-6} M). The EC_{50} values were fairly constant in the different vascular regions tested (Table 1). The contractile response of NPY could not be blocked by phentolamine (10^{-6} M), rauwolscine ($5x10^{-7}$ M), atropine (10^{-6}M), or TTX ($5x10^{-7}$ M).

TABLE 1. Mean values ± SEM for half maximum contraction in vascular areas where direct contractile effects of NPY were tested quantitatively under standardized conditions.

Species	Vessel tested	EC_{50} value (M)	Corresponding pD_2 value	n
Man	Mesenteric artery	$(4.3 \pm 2.2) \times 10^{-8}$	7.44 ± 0.14	4
	Mesenteric vein	$(2.4 \pm 0.1) \times 10^{-8}$	7.75 ± 0.20	5
	Temporal artery	7.0×10^{-9}	8.15	2
Cat	Middle Cerebral artery	$(7.6 \pm 2.5) \times 10^{-9}$	8.29 ± 0.15	8
Rabbit	Auricular artery	$(1.9 \pm 1.1) \times 10^{-7}$	7.41 ± 0.35	8
	Mesenteric artery	$(4.1 \pm 1.1) \times 10^{-8}$	7.47 ± 0.11	6

Interaction between NPY and NE

The possibility that the presence of NPY affected the contractile response to NE at the level of the vascular smooth musculature was tested on the vessels listed in Table 2. Various amounts of NPY ($5x10^{-10}$, 10^{-9}, 10^{-8}, and 10^{-7} M) were added to the bath and were present throughout tests with the series of different NE concentrations. NPY did not cause any consistent change in the cr curve for NE at any of the peptide levels tested: if a change occurred at all it comprised either a slight potentiation or a slight inhibition of the NE response (Fig. 2).

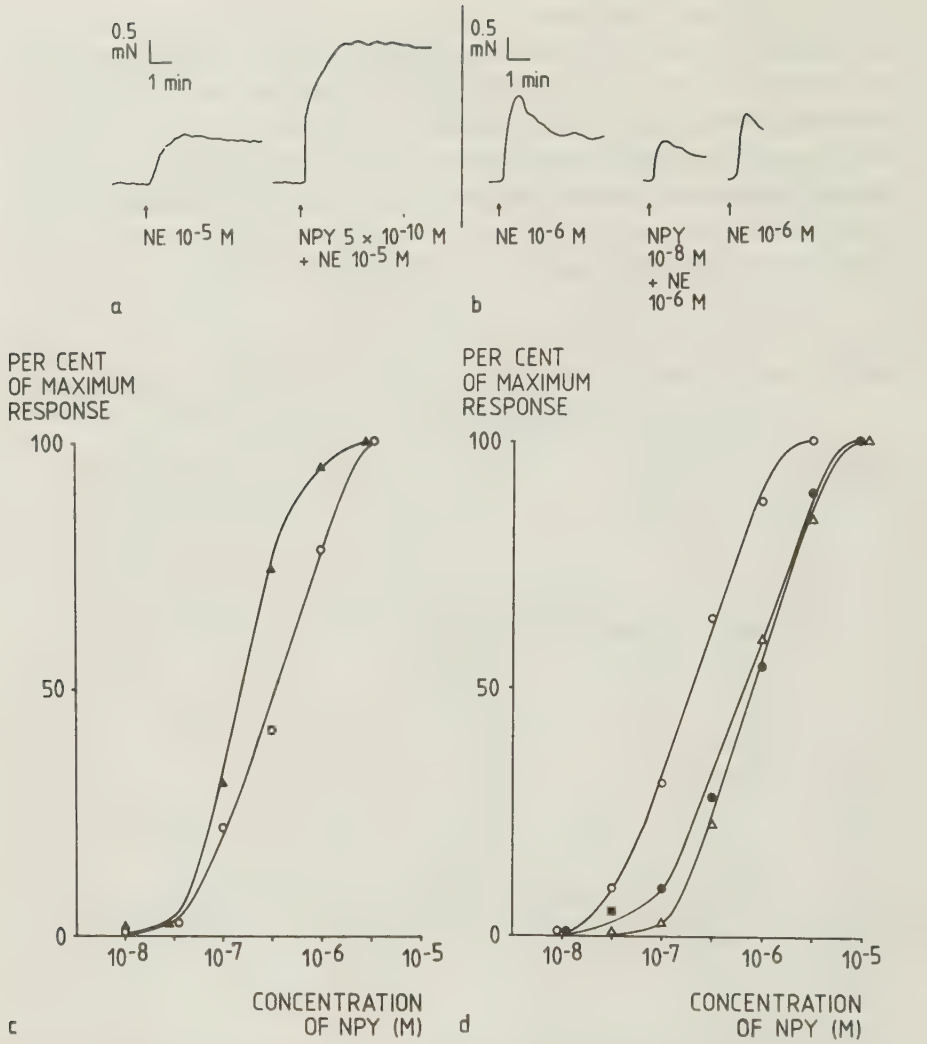


Fig. 2. Examples of NE-induced vasoconstriction in different vascular beds in the presence of NPY. (a) Rabbit gastro-epiploic artery. The effect of 10^{-5} M NE is potentiated by 5×10^{-10} M NPY. (b) Rabbit gastro-epiploic artery. The effect of 10^{-6} M NE is inhibited by 10^{-8} M NPY. (c) Human gastro-epiploic artery showing examples of full cr curves for NE before (o) or after addition of 10^{-7} M NPY (▲), resulting in a slight potentiation. (d) Human mesenteric artery. Full cr curves to NE before (o) and after addition of NPY at concentrations of 5×10^{-10} M (●) or 10^{-8} M (Δ). In this example the effect of NPY was inhibition, which was the same at both NPY-concentrations.

The results were not influenced when NPY was administered to the bath less than 10 min (down to 3 min) before the NE response curve was started. This indicates that inactivation of NPY in the bath was probably not responsible for the lack of shift in the cr curves. Quantitative evaluation of the effect was performed in several types of vessels using an NPY concentration of 10^{-8} M. It can be seen from Table 2 that there was no statistically significant differences in the pD_2 values for NE in the presence or absence of NPY.

TABLE 2. Comparison of mean pD_2 values (± SEM) for NE in the absence or presence of 10^{-8} M NPY based on full cumulative concentration-response curves for the amine.

Species	Vessel tested	pD_2 value for NE			
		Without NPY	n	With 10^{-8}M NPY	n
Man	Mesenteric artery	6.23 ± 0.08	17	6.24 ± 0.07	23
	Mesenteric vein	6.53 ± 0.08	15	6.75 ± 0.08	22
	Gastroepiploic artery	6.56 ± 0.12	5	6.65 ± 0.19	4
Cat	Mesenteric artery	5.76 ± 0.09	9	6.01 ± 0.10	6
Rabbit	Auricular artery	6.24 ± 0.12	5	6.21 ± 0.17	6
	Mesenteric artery	6.03 ± 0.21	10	5.99 ± 0.25	8
	Mesenteric vein	6.83 ± 0.05	3	6.43 ± 0.14	3
	Gastroepiploic artery	6.08 ± 0.09	16	6.24 ± 0.17	14

Note: No significant difference before and after NPY was found for any of the vessels (Student's t-test: $p>0.05$)

Effect of NPY on electrically induced vasoconstriction

It has previously been shown that a purely neurogenic vasoconstriction is not possible to obtain during transmural electrical stimulation of pial arteries (Hardebo et al., 1985). Only peripheral vessels (arteries as well as veins) were therefore tested with regard to the interaction of NPY with the neurally induced vasoconstriction.

Tests were performed on 74 vascular segments in which the electrically induced vasoconstriction (at 0.1 msec pulse duration) was blocked by $5x10^{-7}$ M TTX (64 auricular and 3 gastroepiploic artery segments from rabbit, and 2 arterial and 5 venous segments from the human mesenterium; up to 4 segments were obtained from each vascular region). The contractile response was completely blocked by $3x10^{-6}$ M phentolamine and by 10^{-6} M guanethidine. In 73% of the experiments NPY potentiated the vasoconstriction (Fig. 3) in a concentration-dependent manner (Fig. 4). Only changes of more than 10% of the response in the absence of NPY was considered as a clear-cut effect. No potentiation was seen in the remainder of the vessels.

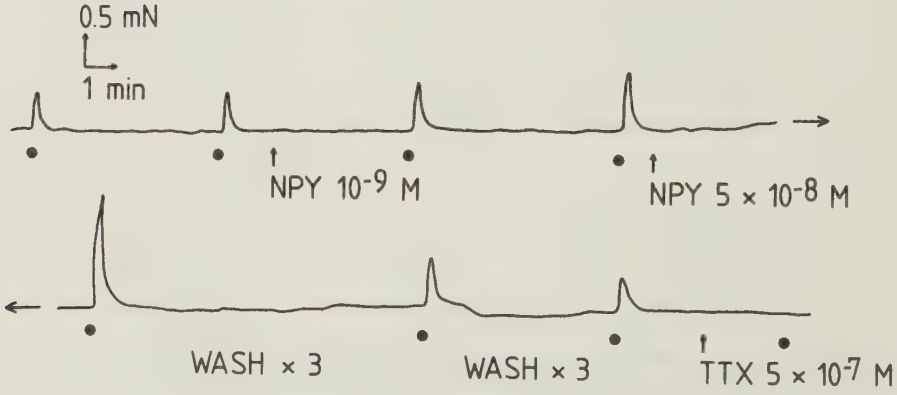


Fig. 3. Example with rabbit central ear artery showing contraction upon electrical field stimulation as marked (9 V, 8 Hz, 0.1 msec pulse duration). NPY (10^{-9} or $5x10^{-8}$ M) potentiated the contraction in a concentration dependent manner, whereas the contraction by NPY on its own was slight. The potentiating effect of NPY disappeared after washing. The electrically induced contraction was blocked by tetrodotoxin (TTX).

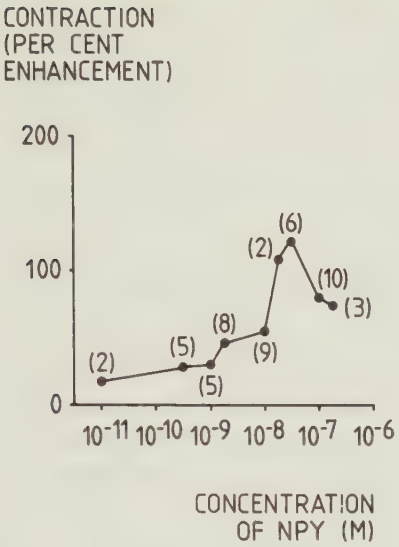


Fig. 4. The mean contraction induced by electrical field stimulation of rabbit´s auricular arteries was potentiated in a concentration-dependent manner by NPY until a maximal effect was reached at a concentration of $5x10^{-8}$ M. Number of experiments within parenthesis.

In six additional experiments (2 basilar, 2 gastroepiploic, and 2 auricular artery segments from rabbit) the vasoconstriction was induced at 0.3 msec pulse duration and could not be blocked by $5x10^{-7}$ M TTX. Also this type of response was potentiated by NPY (Fig. 5).

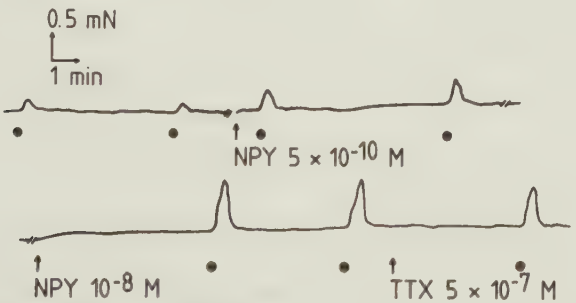


Fig. 5. Example with rabbit´s basilar artery showing contractile response to electrical field stimulation as marked (9 V, 10 Hz, 0.3 msec pulse duration). The contraction could not be blocked by tetrodotoxin (TTX). This non-neurogenic contractile effect (cf. Fig. 3) was enhanced by $5x10^{-10}$ or 10^{-8} M NPY in concentration-related manner.

Discussion

The recently described ability of NPY to cause vasoconstriction in intracranial and peripheral vessels from various animals (Lundberg et al., 1982; Edvinsson et al., 1983; Owman et al., 1984; Hardebo et al., 1985) has in the present study been confirmed and extended to human pial arteries and mesenteric vessels. In previous as well as present experiments it has been shown that the NPY-induced vasoconstriction is not mediated by postsynaptic alpha-adrenergic, serotonergic (5-HT_2), or cholinergic (muscarinic) receptors. However, the vasoconstriction is blocked by the calcium entry blocker, nimodipine (Edvinsson et al., 1983).

In immunocytochemical studies combined with sympathetic denervation it has been demonstrated that NPY occurs together with tyrosine hydroxylase and dopamine-beta-hydroxylase in a population of peripheral adrenergic nerves (Lundberg et al., 1982; Ekblad et al., 1984; Stjernquist et al., 1985). On the basis of this coexistence it might be assumed that NPY and NE interact at the level of the postjunctional receptor. A slight, but statistically significant, potentiation of the NE-induced contraction by NPY has been described for rabbit's gastro-epiploic and femoral arteries (Edvinsson et al., 1984a). In experiments where we tested single doses of NE in the presence of NPY it was sometimes possible to demonstrate a potentiation of the contractions in the same way as described by Edvinsson et al. (1984a) and Ekblad et al. (1984). However, the effect was not reproducible in our hands, not even when tests were repeated on one and the same vascular segment. The same inconsistent potentiation was seen at different NPY concentrations and at various time intervals of NPY exposure (3-10 min) under which the test with NE was performed. In agreement with this, statistical evaluation of experiments with 10^{-8} M NPY, performed on eight different types of vessels from man, cat, and rabbit, did not reveal any significant difference in the pD_2 value for NE in the presence or absence of NPY. It thus seems questionable if a slight potentiation of this kind by NPY, whether statistically significant or not, at all reflects a functionally significant biological phenomenon. Our observations are consistent with findings by Lundberg et al. (1982) on rat's vas deferens, in which $2x10^{-7}$ M of NPY failed to modify the contractile response to $6x10^{-6}$ M exogenous NE. Similar findings have been reported for the portal vein (Lundberg et al., 1984).

Electrical field stimulation of peripheral vessels (mainly rabbit auricular artery) induced a vasoconstriction that was of neurogenic origin, i.e. blockable with TTX when the stimulation current was lower than 10 V and the pulse duration less than 0.15 msec. That the response resulted from activation of adrenergic-type nerves was confirmed by the total blockade with phentolamine and guanethidine (see also Hardebo et al., 1985). Addition of NPY clearly augmented this nerve-induced vasoconstriction in both human and animal vessels. This might indicate a presynaptic potentiation by NPY, since most of the nerve-mediated vasoconstriction is inhibited by an alpha-receptor antagonist (Hardebo et al., 1985) and, as discussed above, NPY has little or no effect on the contractile response to exogenous NE. Direct evidence for presynaptic actions of NPY have been reported by Lundberg and Stjärne (1984) studying the ^{3}H-NE release and contractile response evoked by electrical field stimulation of rat's vas deferens. On the other hand, Ekblad et al. (1984) failed by 5×10^{-7} M NPY to influence the spontaneous or nerve-activated release of tritium from rabbit's gastroepiploic artery labelled with ^{3}H-NE. In the vas deferens and seminal vesicle of rat and guinea-pig NPY inhibits the neurally evoked contractile response in a system where the nature of the motor transmitter is still a matter of controversy (Lundberg et al., 1982; Stjernquist et al., 1985). Also in the uterus a nerve-mediated motor inhibition by NPY has been demonstrated, probably involving cholinergic fibres (Stjernquist et al., 1983).

Experiments carried out with stimulation parameters that resulted in a TTX-resistant vasoconstriction also showed a potentiation by NPY in our study. It is possible that this phenomenon was due to postsynaptic potentiation by NPY of a constrictory activity, induced through some substance released in spite of the nerve conduction block. It has been described that local synaptic depolarization can occur in the presence of TTX (Katz and Miledi, 1967). The substance, however, does not seem to be identical with NE, since NPY did not result in any significant potentiation of the constriction evoked by this transmitter. An alternative explanation for the NPY-induced potentiation in the presence of TTX is that the peptide, through its own constrictory activity, induced alterations in the contractile properties of the smooth muscle cells, such as a slight change in the calcium distribution. This would be enough to potentiate the constriction elicited by the depolarization of the smooth muscle membrane caused by the electrical field. Since the contractile response obtained by Ekblad et al.

(1984) in the rabbit gastroepiploic arteries during electrical field stimulation with a pulse duration as long as 0.3 msec at 15 V is mainly non-neurogenic (Hardebo et al., 1985), it can be assumed that the NPY-evoked potentiation described in the absence of a clearly documented TTX blockade (Ekblad et al., 1984) reflects a postsynaptic event not involving adrenergic receptors. It may be of the same nature as the unexpected, above-mentioned potentiation by NPY of the non-neurogenic, TTX-resistant contraction.

It is conceivable that the coexistence of NPY and NE in the same neurons means that they both are releasable during nerve activation. The present data indicate that NPY interferes with the further release of NE through a presynaptic mechanism rather than a postsynaptic interaction between the two substances.

Adrian TE, Allen M, Bloom SR, Ghatei MA, Rossor MN, Roberts GW, Crow TJ, Tatemoto K, Polak JM (1983) Neuropeptide Y distribution in human brain. Nature 306:584-586

Edvinsson L, Nielsen KC, Owman Ch (1974) Influence of initial tension and changes in sensitivity during amine-induced contraction of pial arteries in vitro. Arch int Pharmacodyn 208:235-242

Edvinsson L, Emson P, McCulloch J, Tatemoto K, Uddman R (1983) Neuropeptide Y: Cerebrovascular innervation and vasomotor effects in the cat. Neurosci Lett 43:79-84

Edvinsson L, Ekblad E, Håkanson R, Wahlestedt C (1984a) Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. Br J Pharmacol 83:519-525

Edvinsson L, Emson P, McCulloch J, Tatemoto K, Uddman R (1984b) Neuropeptide Y: Immunocytochemical localization to and effect upon feline pial arteries and veins in vitro and in situ. Acta Physiol Scand 122:155-163

Ekblad E, Edvinsson L, Wahlestedt C, Uddman R, Håkanson R, Sundler F (1984) Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. Regulat Pept 8:225-235

Everitt BJ, Hökfelt T, Terenius L, Tatemoto K, Mutt V, Goldstein M (1984) Differential co-existence of neuropeptide Y (NPY)-like immunoreactivity with catecholamines in the central nervous system of the rat. Neuroscience 11:443-462

Furness JB, Costa M, Emson PC, Håkanson R, Moghimzadeh E, Sundler F, Taylor IL, Chance RE (1983) Distribution, pathways and reactions to drug treatment of nerves with neuropeptide Y- and pancreatic polypeptide-like immunoreactivity in the guinea-pig digestive tract. Cell Tissue Res 234:71-92

Hardebo JE, Andersson J, Hanko J, Owman Ch (1984) Purinergic and peptidergic vasomotor mechanisms in the brain. In Vasodilator Mechanisms, edited by PM Vanhoutte , SF Vatner. Basel, Karger, pp 128-137

Hardebo JE, Hanko J, Kåhrström J, Owman Ch (1985) Electrical field stimulation in cerebral and perihperal arteries: A critical evaluation of the contractile response. Submitted to Circulation Res

Hunt SP, Emson PC, Gilbert R, Goldstein M, Kimmel JR (1981) Presence of avian pancreatic polypeptide-like immunoreactivity in catecholamine and methionine-enkephalin-containing neurons within the central nervous system. Neurosci Lett 21:125-130

Hökfelt T, Lundberg J, Lagercrantz H, Tatemoto K, Mutt V, Lindberg J, Terenius L, Everitt BJ, Fuxe K, Agnati L, Goldstein M (1983) Occurrence of neuropeptide Y (NPY)-like immunoreactivity in catecholamine neurons in the human medulla oblongata. Neurosci Lett 36:217-222

Katz B, Miledi R (1967) A study of synaptic transmission in the absence of nerve impulses. J Physiol (Lond) 192: 407-436

Lundberg JM, Hökfelt T, Änggård A, Kimmel J, Goldstein M, Markey K (1980) Coexistence of an avian pancreatic polypeptide (APP) immunoreactive substance and catecholamines in some peripheral and central neurons. Acta Physiol Scand 110:107-109

Lundberg JM, Terenius L, Hökfelt T, Martling CR, Tatemoto K, Mutt V, Polak J, Bloom S, Goldstein M (1982) Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. Acta Physiol Scand 116:477-480

Lundberg JM, Terenius L, Hökfelt T, Goldstein M (1983) High levels of neuropeptide Y in peripheral noradrenergic neurons in various mammals including man. Neurosci Lett 42:167-172

Lundberg JM, Stjärne L (1984) Neuropeptide Y (NPY) depresses the secretion of ^{3}H-noradrenaline and the contractile response evoked by field stimulation, in rat vas deferens. Acta Physiol Scand 120:477-479

Lundberg J, Hua X-Y, Franco-Cereceda A (1984) Effects of neuropeptide Y (NPY) on mechanical activity and neurotransmission in the heart, vas deferens and urinary bladder of guinea-pig. Acta Physiol Scand 121:325-332

Owman Ch, Andersson J, Hanko J, Hardebo JE (1984) Neurotransmitter Amines and Peptides in the Cerebrovascular Bed. In: Neurotransmitters and the Cerebral Circulation, edited by ET MacKenzie, J Seylaz, A Bes. New York, Raven Press, pp 11-38

Stjernquist M, Emson P, Owman Ch, Sjöberg N-O, Sundler F, Tatemoto K (1983) Neuropeptide Y in the female reproductive tract of the rat. Distribution of nerve fibres and motor effects. Neurosci Lett 39:279-284

Stjernquist M, Owman Ch, Sjöberg N-O, Sundler F (1985) Co-existence and co-operation between neuropeptide Y and norepinephrine in nerve fibers of guinea-pig vas deferens and seminal vesicle. Biol Reprod 00:000-000

Sundler F, Moghimzadeh E, Håkanson R, Ekelund M, Emson P (1983) Nerve fibers in the gut and pancreas of the rat displaying neuropeptide Y immunoreactivity. Cell Tissue Res 230:487-493

Tatemoto K (1982) Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. Proc Natl Acad Sci USA 79:2514-2518

Tatemoto K, Carlquist M, Mutt V (1982) Neuropeptide Y - a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. Nature 296:659-660

Terenghi G, Polak JM, Allen JM, Zhang SQ, Unger WG, Bloom, ST (1983) Neuropeptide Y-immunoreactive nerves in the uvea of the guinea pig and rat. Neurosci Lett 42:33-38

Törnebrandt K, Nobin A, Owman Ch (1985) Pharmacological Characterization of α-adrenergic Receptor Subtypes Mediating Contraction in Human Mesenteric Arteries and Veins. Blood Vessels 000:000-000

Uddman R, Edvinsson L, Håkanson R, Owman Ch, Sundler F (1982) Immunohistochemical demonstration of APP (avian pancreatic polypeptide)-immunoreactive nerve fibres around central blood vessels. Brain Res Bull 9:715-718

Uddman R, Sundler F, Emson P (1984) Occurrence and distribution of neuropeptide Y-immunoreactive nerves in the respirtory tract and middle ear. Cell Tissue Res 23:321-327